Influence of first morning urine volume, fasting blood glucose and glycosylated hemoglobin on first morning urinary albumin concentration

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Abstract

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The aim of the present study was to evaluate the effect of first morning urinary volume (collected on three different non-consecutive days), fasting blood glucose (determined on the first and third days of urine collection), and glycosylated hemoglobin (determined on the first and third days of urine collection) on the albumin concentration in first morning urine samples collected on three different days. We found 3.6% asymptomatic bacteriuria in the urine samples; therefore, every urine sample must be tested to exclude infection. One hundred and fifty urine samples were provided by 50 IDDM patients aged 21.9 ± 7 (12-38) years with a disease duration of 6.8 ± 5.8 (0.4-31) years attending the Diabetes Clinic at the State University Hospital of Rio de Janeiro. There were no differences in albumin concentration (6.1 vs 5.8 vs 6.2 μg/ml; P = NS) or urinary volume (222.5 vs 210 vs 200 ml) between the three samples. In addition, there were no differences in fasting blood glucose (181.9 ± 93.6 vs 194.6 ± 104.7 mg%; P = NS) or glycosylated hemoglobin (HbA1) (8.4 ± 1.3 vs $8.8 \pm 1.5\%$; P = NS) between the first and third blood samples. Six patients (group 1) had a mean urinary albumin concentration of more than 20 μg/ml for the three urine samples. This group was compared with the 44 patients (group 2) with a mean urinary albumin concentration for the three urine samples of less than 20 μg/ml. No difference was found between groups 1 and 2 in relation to fasting blood glucose (207.1 ± 71.7 vs 187.6 ± 84.6 mg/dl), HbA1 (8.1 ± 0.9 vs $8.6 \pm 1.1\%$) or urinary volume [202 (48.3-435) vs 246 (77.3-683.3) ml]. Stepwise multiple regression analysis with albumin concentration of first morning urine samples as the dependent variable, and urinary volume, fasting blood glucose and glycosylated hemoglobin as independent variables, showed that only 12% (P = 0.01) of the albumin concentration could be accounted for by the independent effect of morning urine volume on the first day of urine collection. No urine samples showed a change in the cutoff level of 20 μg/ml of albumin concentration as the result of volume. Fasting blood glucose and glycosylated hemoglobin did not influence the urinary albumin concentration. Considerable variability in urinary albumin concentration was found in the three morning urine samples with a mean intraindividual coefficient variation of 56%. In conclusion, in the present study, urinary volume had a minimal, though not constant, effect on first morning urinary albumin concentration. Day-to-day metabolic and clinical control of IDDM patients, except probably for ketoacidosis, should not contraindicate microalbuminuria screening in first morning urine samples.

Introduction

Diabetic nephropathy is the main cause of increased morbidity and mortality in type I (insulin-dependent) diabetes mellitus (IDDM), affecting at least one-third of young diabetic subjects (1). A urinary albumin excretion rate (AER) above normal levels (microalbuminuria) but as yet undetectable by standard laboratory methods seems to be an early predictor of the development of nephropathy (2). These observations have been used to formulate the concept of incipient nephropathy (3). Microalbuminuria is not only related to diagnosis of incipient nephropathy but is also important for the early prevention of overt nephropathy (4) and to predict mortality from cardiovascular disease (5). The present level of increased AER has been set at 20 µg/min or 30 µg/min in at least two consecutive urine collections, with the "timed 24 h" and "overnight" urine collections being currently proposed (3). However, for screening purposes, untimed collections are advantageous. Most studies have dealt with the usefulness of the albumin concentration or the albumin/creatinine ratio in a random or morning urine collection for first screening (6-9). However, urine volume and patient metabolic control could interfere with this measurement.

The aim of the present study was to determine the effect of first morning urine volume, fasting blood glucose and glycosylated hemoglobin (HbA$_1$) on albumin concentration in the first morning urine sample collected on three different non-consecutive days.

Material and Methods

One hundred and ninety-eight first morning urine samples were obtained on three different non-consecutive days from 50 insulin-dependent diabetic outpatients (22 males and 28 females) aged 21.9 ± 7 (12-38) years with a disease duration of 6.8 ± 5.8

(0.4-31) years attending the Diabetes Clinic at the State University Hospital of Rio de Janeiro and classified according to the National Diabetes Data Group (10). All urine samples were obtained from patients under a satisfactory clinical control without any clinical signs or symptoms of diabetes decompensation (polyuria, thirst, weight loss, etc.) or ketoacidosis and on a day-to-day activity level. All patients received written instructions about urine collection and were instructed to maintain their usual diet and physical activity.

All urine samples passed shortly after the patients got up in the morning were collected into a container without a preservative. The urine volume was recorded for these samples and aliquots were separated into glass tubes. On the same day, fresh urine samples were collected for urine culture at the hospital. A fasting blood sample was also obtained on the first and third days of urine collection for glucose and HbA$_1$ determinations.

The urine samples were assayed for urinary albumin concentration within 7 days of storage at 4°C after centrifugation. Albumin concentration was measured by radioimmunoassay (Diagnostic Product Corporation, Los Angeles, CA). This assay had a sensitivity of 0.3 µg/ml and intra-assay and inter-assay coefficients of variation were 2.7% and 3.5%, respectively.

Serum glucose was measured by the glucose oxidase method (Cobas-Mira Roche, Switzerland) and glycosylated hemoglobin by cation-exchange chromatography (Bayer Diagnostic, Germany; reference range, 4.5 to 8%).

Statistical analysis

Since the albumin concentration in first morning urine samples has a skewed distribution, the Mann-Whitney U-test was used for comparisons between two independent samples and the Wilcoxon test for comparisons between two dependent samples. For

comparison between two dependent groups with normally distributed data, the paired Student t-test was used. For comparison between more than two independent groups with normally distributed data, one-way ANOVA was used; otherwise the Kruskal-Wallis test was performed. For comparison between dependent samples not normally distributed with more than two measures, the Friedman test was used. When performing stepwise multiple regression analysis the albumin values were log transformed and were the dependent variable. The independent variables were first morning urinary volume, fasting blood glucose and glycosylated hemoglobin. The analyses were performed using the Statistical Package for the Social Science (SPSS) and EPI INFO (version 6.0). Data are reported as mean ($\pm$ SD) for normally distributed data and median (range) for skewed data. A P value less than 0.05 was considered to be significant. Mean intraindividual coefficients of variation for first morning albumin concentration and volume were also calculated (11).

Results

All three urine samples were collected within six months. Considering the 198 urine specimens, 24.4% (N = 48) had some level of bacteriuria, with 20.8% (N = 41) having less than 100,000/mm^3 but more than 50,000/mm^3 and 3.6% (N = 7) having more than 100,000/mm^3 of bacteriuria. These 48 urine samples were discarded. Therefore, only the results for 150 urine specimens were submitted to statistical analysis.

The mean intraindividual coefficients of variation for urinary volume and albumin concentration in the first morning urine between the three samples were 68.2% and 56%, respectively. The laboratory data for urinary albumin, urinary volume, fasting blood glucose and HbA$_1$ are shown in Table 1. No differences in albumin concentration or urinary volume were observed between the three samples. In addition, there were no differences in fasting blood glucose or HbA$_1$ between the first and third blood samples. A total of six patients (group 1) had a mean urinary albumin concentration of over 20 µg/ml for the three urine samples. This group was compared with the 44 patients (group 2) with a mean urinary albumin concentration for the three urine samples of less than 20 µg/ml. No difference was found between groups 1 and 2 in relation to fasting blood glucose (207.1 $\pm$ 71.7 vs 187.6 $\pm$ 84.6 mg/dl), HbA$_1$ (8.1 $\pm$ 0.9 vs 8.6 $\pm$ 1.1%) or urinary volume [202 (48.3-435) vs 246 (77.3-683.3) ml].

Stepwise multiple regression analysis with albumin concentration as the dependent variable and urinary volume, fasting blood glucose and HbA$_1$ as independent variables was performed with the data obtained on the three different days of urine collection. Urinary volume in the first morning urine sample reached a significant level, whereas fasting blood glucose and HbA$_1$ did not. Therefore, we performed a univariate analysis with albumin concentration as the dependent variable and urinary volume as the independent variable in all urine samples. These results are reported in Table 2. No urine sample with an albumin concentration above 20 µg/ml showed a significant influence of the urinary volume.

Table 1 - First morning urine albumin concentration, volume, fasting blood glucose and HbA$_1$ of 50 IDDM patients.

Data for urinary albumin and first morning urine volume are reported as median (range) (Friedman test). The other data are reported as means $\pm$ SD. There were no statistical differences between samples for any of the variables studied (Student t-test).

Variables	Samples		
	First	Second	Third
Urinary albumin (µg/ml)	6.1 (1-95)	5.8 (1.1-80)	6.2 (1.2-75)
Urine volume (ml)	222.5 (27-910)	210 (5-1120)	200 (25-640)
Glucose (mg/dl)	181.9 ± 93.6		194.6 ± 104.7
HbA$_1$ (%)	8.4 ± 1.3		8.8 ± 1.5

Table 2 - Univariate regression analysis of first morning urinary albumin concentration with first morning urine volume of 50 IDDM patients.

Urinary volume	First urinary albumin concentration			
	R^2	F (ANOVA)	ß Coefficient	P value
First sample	0.12	6.74	-6.83 E04	0.01
Second sample	0.05	2.64	-3.8 E04	0.1
Third sample	0.08	4.33	-5.9 E04	0.04

Discussion

Early identification of microalbuminuria is considered to be clinically important although some relevant issues concerning the methodology remain unresolved as recently discussed in Ref. 3. This fact is very important because many reports have demonstrated that therapeutic intervention can delay the development of end stage renal disease (12). Therefore, the precise classification of diabetic patients with respect to microalbuminuria or normoalbuminuria is important clinically.

In the present study, although only 7 (3.6%) urine samples presented bacteriuria more than 100,000/mm^3, without any symptoms of urinary tract infection, we have discarded all urine samples with bacteriuria more than 50,000/mm^3 because there are no studies about the correlation between the level of increase in microalbuminuria and the level of asymptomatic bacteriuria. No increase or a small increase in albumin concentration has been described in non-insulin-dependent diabetic patients or in the non-diabetic subjects with symptomatic bacteriuria (13). Recently, 6% of bacteriuria more than 100,000/mm^3 has been found in a randomly selected subset of 33 diabetic patients. These subjects, aged more than 60 years, were not excluded from screening for microalbuminuria (14). Our sample was comprised of younger patients and only those with IDDM. Thus, until the contribution of bacteriuria is clarified, we must perform a clinical as well as a laboratory investigation of urinary tract infection in all urine collections for microalbuminuria determination.

The effect of first morning urine volume on albumin concentration was confirmed in the present study. Univariate analysis demonstrated that no more than 12% of the variance in first morning urinary albumin concentration could be accounted for by the independent effect of urine volume. We emphasize that this is a correlation of low intensity with a low beta coefficient. We did not find a significant alteration in the level of urinary albumin concentration that would modify the conventional cutoff level of 20 µg/ml used to predict an albumin excretion rate of more than 20 or 30 µg/min in timed urine collections (3). Therefore, the variability of urinary volume should not result in the misclassification of diabetic patients for screening purposes. Some reports have described the possible influence of volume on albumin concentration in first morning or overnight urine samples (9,14,15) and, although we agree with them, the variability which we have found was minimal and not constant for our patient sample.

In the present study we have confirmed that metabolic control measured by fasting blood glucose and glycosylated hemoglobin on the same morning of urine collection did not influence urinary albumin concentration. Few studies evaluating the influence of metabolic control on urinary albumin concentration during urine collections have been performed. In one study in which insulin was reduced or withdrawn for some days in IDDM patients the mean albumin excretion rate increased 16 ± 5.7 mg/24 h while urine volume remained constant (16). However, in the day-to-day management of diabetic patients, no influence on the level of urinary albumin excretion by fasting blood glucose or urinary glucose excretion (17,18) during the day of urine collection has been observed. We did not find any difference be-

tween groups 1 and 2 in fasting blood glucose, HbA_1 or urinary volume. Since patients with a mean albumin concentration of more than 20 μg/ml in first morning urine samples have a greater probability of being microalbuminuric (19), we may infer that we did not find any difference between normo- and microalbuminuric patients.

The metabolic control performed a few weeks before did not influence the level of urinary albumin concentration as shown by the unchanged levels of HbA_1 in the blood samples obtained on two different days. A majority of cross-sectional studies have shown the same results (17,18,20). A cross-sectional study involving non-insulin diabetic patients has demonstrated that fasting blood glucose higher than or equal to 160 mg/dl was significantly and independently associated with the development of proteinuria (21). However, the level of metabolic control after diabetes diagnosis was the most important variable for the development of microalbuminuria and macroalbuminuria as many prospective studies have shown (4,22,23).

A mean intraindividual coefficient of variation in urinary albumin concentration of 56% was observed in the present study. A level between 30 to 50% has been found in several studies (11,18,20). Thus, urinary volume, fasting glucose or glycosylated hemoglobin alone cannot account for this variability. Probably individual biological variations seem to be the most important factors (9,11,18,20).

In conclusion, the influence of first morning urine volume on urinary albumin concentration is minimal and not constant. Furthermore, the level of day-to-day metabolic and clinical control in IDDM patients, except probably for patients with ketoacidosis, should not contraindicate microalbuminuria screening in a first morning urine sample. This fact is very important for IDDM outpatients attending public hospitals in developing countries where optimal diabetic control may be very difficult to achieve.

Acknowledgment

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