

Research article

PHYSICAL ACTIVITY ALTERS URINARY ALBUMIN/ CREATININE RATIO IN TYPE 1 DIABETIC PATIENT

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ABSTRACT

While the best way to identify microalbuminuria is to determine albumin excretion rate (AER) in a 24 h urine sample. Published data have shown that calculation of an albumin/creatinine ratio (ACR) in a spot urine sample has reasonable rate of sensitivity and specificity. We aimed to evaluate the effect of daily exercise on ACR and estimate the best time for the examination of the ACR in a spot urine sample. Sixteen eligible patients with Type 1 diabetes mellitus were asked to perform varying degree of exercise periods. Urinary albumin and creatinine excretion rates during each period were determined. ACR and AER of timed urinary samples were compared with the 24 hour urinary AER. We found significant correlations between timed and 24 hour urinary AER. According to diagnostic performance tests, ACR and AER of timed urine samples were both found to be significantly more sensitive during resting period when compared with mild or moderate active periods. It is concluded that ACR and AER of a timed urine sample are sensitive and specific methods for determining microalbuminuria, while overnight resting samples give the impression of being more diagnostic.

KEY WORDS: Microalbuminuria, albumin/creatinine ratio, type 1 diabetes mellitus, exercise, nephropathy.

INTRODUCTION

The presence of microalbuminuria (30-299 mg·dL⁻¹ or 20-199 µg·min⁻¹) predicts deterioration of renal disease to overt diabetic nephropathy in diabetic patients (Viberti et al., 1982; Krolewski et al., 1985) and elevated risk of cardiovascular disease in diabetic and non-diabetic individuals (Agewall et al., 1997; Yudkin et al., 1988). The early detection of microalbuminuria has been widely advocated as means of diagnosing and treating early renal involvement in diabetes (MCSG, 1993; Bennett et al., 1995). Measurement of albumin excretion rate (AER) in a 24 hour urine sample is the most reliable way for the detection of microalbuminuria (Mogensen et al., 1995; Tobe et al., 2002). Quantitative or qualitative assessment of AER in a spot urine sample gives high rate of false negative

and positive results due to water intake. Published data have shown that the calculation of albumin/creatinine ratio (ACR) in a spot urine sample exhibits a higher sensitivity and specificity in the detection of microalbuminuria (Gatling et al., 1985; Eshoj et al., 1987; Khawali et al., 2002). This method has certain advantages, as it is easily available, non-expensive and reproducible, but several conditions such as posture, exercise, ketosis may lead to inappropriate increase of the urinary albumin excretion (Abbott et al., 1994; Gomes and Goncalves, 2001).

The value of ACR is controversially discussed in the literature. Whereas some authors recommended ACR as the method of choice for the detection of microalbuminuria (Shield et al., 1995; Newman et al., 2000; Tobe et al., 2002), other published data demonstrated only a limited value of

Table 1. Clinical and laboratory characteristics of the patients. Data are mean (SD).

	Normoalbuminuria	Microalbuminuria
N	9	7
Age (y)	25.2 (3.6)	29.1 (3.4)
Gender (Men/Women)	3/6	2/5
Diabetes duration (y)	7.5 (3.6)	8.7 (5.8)
BMI (kg·m ⁻²)	22.1 (3.3)	23.0 (2.7)
Insulin dose (U·d ⁻¹)	42.7 (23.7)	49.4 (19.6)
Systolic BP (mmHg)	111 (6)	115 (7)
Diastolic BP (mmHg)	71 (5)	76 (5)
Serum creatinine (mg·dL ⁻¹)	0.82 (0.19)	0.77 (0.19)
HbA1c (%)	10.1 (1.4)	9.5 (1.6)

ACR as a screening method of microalbuminuria (Marshall, 1991; Derhasching et al., 2002). The circadian rhythm of proteinuria, blood glucose control, dietary protein content and physical activity are the main contributors of the inaccuracy of ACR (Rowe et al., 1984; Jefferson et al., 1985). In this study we aimed to evaluate the effect of daily exercise on ACR and estimate the best time and best intensity of the physical activity for the examination of the ACR in a spot urine sample to detect microalbuminuria in patients with Type 1 diabetes mellitus.

METHODS

Patient selection

Patients with Type 1 diabetes mellitus without hypertension, macroalbuminuria or renal insufficiency. Insulin treated diabetic patients with the onset of diabetes before the age of 25, with duration of diabetes more than 3 years and with no clinical evidence of cardiovascular, peripheral vascular or renal disease were screened for AER. Patients with the history of trauma, operation, ketoacidosis, hypoglycemia led to unconsciousness in last 4 weeks or using medication except insulin were not included into the study. Spot urine specimens were tested for gross proteinuria by means of a reagent strip (Labstix, Ames, Miles Inc., Elkhart, IN, USA) and patients with gross proteinuria and abnormal urine sediments were excluded.

Patients were hospitalized to our medical center and they were instructed to collect 24 hour urine specimens without any extra exercise other than instructed. Patients were included into the study if their blood pressure, AER and serum creatinine levels were less than 140/90 mmHg, 200 $\mu\text{g}\cdot\text{min}^{-1}$, 1.4 $\text{mg}\cdot\text{dL}^{-1}$, respectively. The study population consisted of 9 patients with AER less than 20 $\mu\text{g}\cdot\text{min}^{-1}$ and 7 patients with AER more than 20 $\mu\text{g}\cdot\text{min}^{-1}$ but less than 200 $\mu\text{g}\cdot\text{min}^{-1}$. Table 1 shows some of the clinical and laboratory characteristics of

the patients in which no statistical difference was established between any characteristic. Patients gave written informed consent to the study, which was approved by Ethics Committee of Uludag University.

Urine sampling and analysis

Patients were asked to perform 4 periods with different physical activity intensities. Exercise intensities were aimed to be in accordance with daily activities which were validated with pedometer. In the first period (Mild I), they stepped between 1000-5000 in 4 hours (08:00-12:00). In the second period (Moderate) they were asked to walk more than 10 000 steps in 4 hours (13:00-17:00) and to be on upright position. In the third period (Mild II) they were again asked to walk approximately 1000-5000 steps in 4 hours (18:00-22:00) and in the fourth period (Resting) they were requested to lay down as much as possible and to have step count less than 500 (23:00-08:00). Timed urine samples were collected separately by 1 hour further at the end each period. Blood glucose levels were checked in every hour during the third period and at 3 hour intervals during rest of the day to exclude hypoglycemia and striking hyperglycemia.

Urinary albumin and creatinine excretion rates of urine samples were determined. Timed urinary AER ($\mu\text{g}\cdot\text{min}^{-1}$) and ACR ($\mu\text{g}\cdot\text{mg}^{-1}$) of each period were calculated with the contribution of albuminuria, creatininuria and urine volumes. Urinary albumin level was measured using a competitive immunoassay (Immulite[®], DPC, Los Angeles, CA, USA) with interassay and intra-assay variations of 4.4% and 6.7% respectively. Urinary creatinine concentration was analyzed by the Jaffe reaction. Automated biochemical analysis was used for the other determinations in plasma.

Statistical analysis

The albumin concentration measured in the timed urine samples were compared with the albumin concentration quantified from 24-h urine sample of the previous day. AER less than 20 $\mu\text{g}\cdot\text{min}^{-1}$ in the

Table 2. Sensitivity, specificity, positive and negative predictive rates (%) of ACR less than 26 $\mu\text{g}\cdot\text{mg}^{-1}$ during different intensities of physical activity for demonstrating normoalbuminuria.

	Mild I	Moderate	Mild II	Resting	Total
Sensitivity	71	67	100	86	80
Specificity	78	70	81	89	79
Positive predictive	71	57	71	86	71
Negative predictive	78	78	100	89	86

24-h urine sample was considered as normoalbuminuria. Relationships between 24-h urine samples and timed urine specimens were sought by Pearson correlation. Cut-off values of ACR and AER in timed urine samples were calculated by insertion of 95% confidence intervals to the mean levels of patients with normoalbuminuria quantified from 24-h urine sample. Diagnostic performance was expressed in terms of specificity, sensitivity, positive and negative predictive value. Data are given as mean and standard deviation (SD). Statistical analyses were performed with SPSS 10.0 / PC (SPSS Inc., Chicago, IL), and statistical measures were calculated in the standard manner.

RESULTS

All patients completed the exercising periods within the target step levels. There were no major adverse events during this study. Any patient did not have any symptoms resembling hypoglycemia. Patients blood glucose levels were ranged between 72-282 $\text{mg}\cdot\text{dL}^{-1}$. Patients with blood glucose levels higher than 200 $\text{mg}\cdot\text{dL}^{-1}$ were checked for ketonuria which nobody presented.

Pearson's correlations between 24-h urinary AER and urinary ACR are shown in Figure 1. Scatter plot diagrams depict the significant relationship between 24-h urinary AER and urinary ACR during all the periods. In according to calculate the diagnostic performance of ACR, mean range with %95 confidence interval was used as the cut point. The mean level of ACR in 9 patients with normoalbuminuria was 17 $\mu\text{g}\cdot\text{mg}^{-1}$ with a 95% confidence interval 9 $\mu\text{g}\cdot\text{mg}^{-1}$. Twenty six $\mu\text{g}\cdot\text{mg}^{-1}$ (17 $\mu\text{g}\cdot\text{mg}^{-1}$ + 9 $\mu\text{g}\cdot\text{mg}^{-1}$) was accepted as the cut

point for detecting normoalbuminuria for using ACR in spot urine samples. According to this cut off value of ACR, diagnostic performance was compared against microalbuminuria results detected by 24-h urine samples (Table 2). Most predictive results were obtained during resting and mild physical activity periods.

Similar calculations were done for urinary AER determined in timed urine specimens during different periods (Figure 2). In patients without microalbuminuria cut point for urinary AER in timed urinary specimens was found as 15 $\mu\text{g}\cdot\text{min}^{-1}$ (12 $\mu\text{g}\cdot\text{min}^{-1}$ + 3 $\mu\text{g}\cdot\text{min}^{-1}$) and diagnostic performance of the AER for this cut-off value during different periods were calculated (Table 3). Diagnostic performance of urinary AER determined in timed urinary specimens showed very similar percentages with ACR. When effect of intensity of exercise on AER considered, resting and mild physical activity seem to be the most accurate.

DISCUSSION

Early detection of microalbuminuria through screening allows interventions aimed at preventing diabetic nephropathy (Mogensen and Chritensen, 1984; Borch-Johnsen et al., 1993). Pathologic microalbuminuria was assumed when the albumin concentration exceeded 20 $\mu\cdot\text{min}^{-1}$ in a 24-h urine sample. Because collection of 24-h urine is cumbersome for the patients, many authors claimed that spot urine samples can give reliable results for detecting microalbuminuria. The best method to diagnose microalbuminuria in a spot urine sample is still a matter of discussion. Qualitative measurement of albuminuria in a spot urine sample can give very

Table 3. Sensitivity, specificity, positive and negative predictive rates (%) of AER less than 15 $\mu\text{g}\cdot\text{min}^{-1}$ in the timed urine specimens during different intensities of physical activity for demonstrating normoalbuminuria.

	Mild I	Moderate	Mild II	Resting	Total
Sensitivity	50	57	100	88	71
Specificity	67	67	90	100	81
Positive predictive	71	57	86	100	79
Negative predictive	44	67	100	89	75

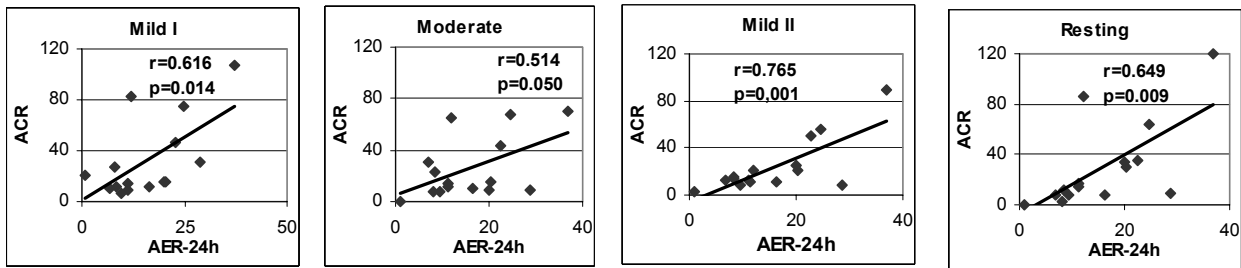


Figure 1. Pearson's correlation between 24 hour urinary AER ($\mu\text{g}\cdot\text{min}^{-1}$) and ACR of urine specimens ($\mu\text{g}\cdot\text{mg}^{-1}$) collected during different physical activity intensities.

high levels of false positive/negative results depending on the volume of urine excreted that can be highly variable depending mainly on the individual's fluid intake and physical activity (Mckenna et al., 1991; Carella et al., 1994; Newman et al., 2000). Proteinuria detected by using dipstick urinalysis despite correlates well with radioimmunoassay measurement also documented many false positive values and necessitate repeated testing (Pegoraro et al., 1997). Our results also showed low levels of sensitivity and specificity when quantitative measurement of albuminuria in the urine samples compared with 24-h urine microalbuminuria measurements (data not shown). Timed urine collections, ACR in spot urine and early morning urine are other alternative specimens for the measurement of albumin excretion.

Our results demonstrated a strong correlation between AER in the timed urine specimens and 24-h urinary AER. We aimed to detect the best cut-off value of timed urine sample for the limit of microalbuminuria. In patients without microalbuminuria the cut-off value was calculated as $15 \mu\text{g}\cdot\text{min}^{-1}$ for timed urine specimens. So, it is assumed that in a timed urine sample albumin excretion less than $15 \mu\text{g}\cdot\text{min}^{-1}$ shows normoalbuminuria with 95% confidence. Sensitivity and specificity rates of AER level $< 15 \mu\text{g}\cdot\text{min}^{-1}$ in a timed urine specimen for detecting normoalbuminuria were found 71% and 82% respectively.

Our foremost aim was to identify the best time for collecting spot urine sample in detecting

microalbuminuria correctly. When diagnostic performance tests were made for the timed urine samples by considering $15 \mu\text{g}\cdot\text{min}^{-1}$ as the cut-off value, it was found that increased physical activity led to low percentage of sensitivity and specificity. For this cut-off value sensitivity and specificity values during the resting period were 88% and 100% respectively, but in moderately active period these rates were 57% and 67% respectively. So it was concluded that for the detection of urinary albumin excretion accurately in a timed urine sample, inactivity is important.

In the resting period patients were instructed to be inactive as much as they could. Sensitivity and specificity rates of the third period with mild physical activity were very close to the resting period. Since the third period activity (Mild II) was similar with the first period (Mild I), it is obvious that physical activity is not the only cause in the variation of urinary albumin excretion. Blood pressure, sodium intake, protein intake, hyperglycemia could be factors for low sensitivity and specificity of the daytime urine samples.

Timed collections are too cumbersome for the patients and comprise potency for erroneous results during the collection of the timed urine sample. In clinical practice it is very difficult to verify that a timed collection of urine is complete, so urinary albumin measurement in timed urine sample leads to considerable percentage of falsely positive/negative results. Spot urine samples which can easily be acquired in the ambulatory setting has many advantages. To evaluate the diagnostic performance

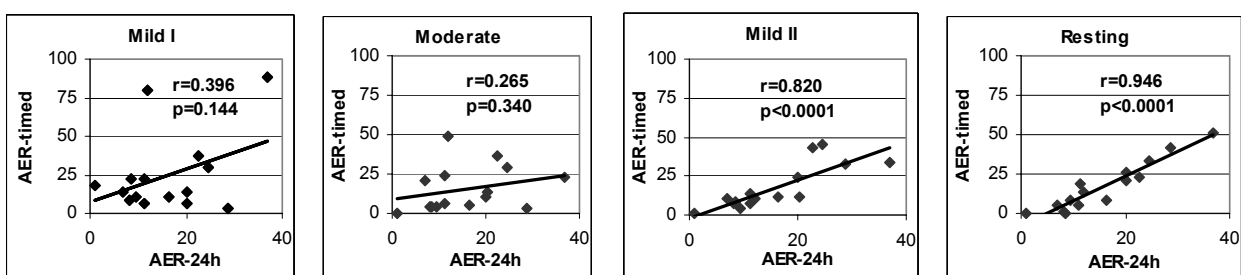


Figure 2. Pearson's correlation between 24 hour urinary AER ($\mu\text{g}\cdot\text{min}^{-1}$) and urinary AER of timed urine specimens ($\mu\text{g}\cdot\text{min}^{-1}$) collected during different physical activity intensities.

of the albumin excretion in a spot urine sample, we calculated ACR for every period urine samples and compared with the results of urinary albumin excretion results of the 24-h urine samples.

Correlation between albumin/creatinine ratio and urinary albumin excretion of 24-h urine sample was very significant. To find the best cut-off value for results of albumin/creatinine ratio mean levels and 95% confidence intervals were evaluated. In patients without microalbuminuria calculated cut-off value was $26 \mu\text{g}\cdot\text{mg}^{-1}$ and this level was evaluated for the significance of diagnostic performance for different periods. Sensitivity and specificity rates of ACR level $< 26 \mu\text{g}\cdot\text{mg}^{-1}$ for detecting normoalbuminuria were 80% and 79%, respectively. When diagnostic performance tests were performed for each period, best results were obtained during resting period with sensitivity rate of 86% and specificity rate of 89%.

CONCLUSIONS

In conclusion although spot urine samples give false positive and negative results, timed urine samples can predict microalbuminuria with a very high accurate percentage if samples collected without erroneously. Our data did not provide any advantage of ACR compared with albuminuria measured in timed urine sample. For timed urine samples albumin excretion less than $15 \mu\text{g}\cdot\text{min}^{-1}$ is highly predictive for normoalbuminuria. In conditions of uncertainty about collection of timed urine sample, albumin creatinine ratio can be used with great confidence. In this condition, albumin creatinine ratio lower than $26 \mu\text{g}\cdot\text{mg}^{-1}$ is well predictive for normoalbuminuria. With increasing physical activity during urine collection diagnostic performances of the cut-off values go downhill. For detecting microalbuminuria best results are reached with the early-morning urine samples.

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KEY POINTS

- Timed urine samples can predict microalbuminuria but because of the erroneous urine collections, microalbuminuria measurement should be calculated with creatinuria measurement.
- With increasing physical activity during urine collection diagnostic performances of the cut-off values go downhill.
- For detecting microalbuminuria best results are reached with the early-morning urine samples.

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