Second Morning ACR Could Be the Alternative to First Morning ACR to Assess Albuminuria in Elderly Population

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Background: Albumin/creatinine ratio (ACR) from a first morning urine is recommended as a early indicator for diabetic nephropathy. However, it is not always feasible to collect the first morning urine for outpatients. We aimed to explore whether ACR from a second morning urine had a good consistency with that from a first morning urine to predict albuminuria in Chinese elderly citizens. **Method:** One hundred and ninety-one elderly citizens (≥ 60 years old) from Junliangcheng community, Dongli district, Tianjin, China were included. A first and second morning urine was collected from each participants, successfully and detected the urinary albumin and creatinine of each urine sample. Albumin to creatinine ratio from a first morning urine (ACR1) was compared with that from a second morning urine (ACR2), and the ability of ACR1 and

ACR2 to predict albuminuria was assessed. **Result:** ACR1 and ACR2 were highly correlated ($r = 0.901$), especially in male and hypertension group ($r = 0.938$ and 0.904). The slope and intercept were 0.93 and 0.11 after log-transformed. And there was no statistical difference between values of ACR1 and ACR2 ($P = 0.271$). Overall, 26.2% participants were detected with albuminuria when judged by ACR1 and 28.3% by ACR2. A good concordance of ACR category (normal or albuminuria) was found between ACR1 and ACR2 (Kappa value = 0.815 in overall; in male and hypertension group were 0.900 and 0.850). **Conclusion:** A second morning urine ACR could be the alternative to a first morning urine ACR for albuminuria detection in elderly population. *J. Clin. Lab. Anal.* 30:175–179, 2016. © 2015 Wiley Periodicals, Inc.

Key words: albumin to creatinine ratio; second morning urine voids; first morning urine voids; albuminuria; elderly population

INTRODUCTION

Increased urinary albumin excretion is recognized as an independent marker for kidney damage (1). It plays an important role in monitoring the progression of diabetic nephropathy and cardiovascular disease (1–3). Recently, many population-based studies (4–7) show a high prevalence of albuminuria in elderly population. So it is relevant to screen and monitor albuminuria in elderly population. Urinary albumin excretion in 24 hr urine collection is recognized as the “gold standard” (8). However, 24 hr urine samples are subject to errors due to missed or improper timing samples and it is difficult for patients to comply. Numerous studies (9–11) recommended albumin to creatinine ratio (ACR) from a first morning void as a surrogate to assess albuminuria.

However, it is not always feasible to collect a first morning urine for outpatients, because most people urinate the

first morning urine at home. A study (12) demonstrated that ACR from a second morning urine could be an alternative to 24 hr urine collection. For outpatients a second morning urine is more convenient to apply. But, until now there is no study to analyze the difference between the first morning urinary ACR (ACR1) and the second morning urinary ACR (ACR2). We aim to explore whether ACR2 has a good consistency with ACR1 when assessing albuminuria in Chinese elderly citizens.

Contribution: Study design: Hua Bin Wang, Rui Liu.

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TABLE 1. Characteristics of the Study Population^a

	Overall (n = 191)	Men (n = 85)	Women (n = 106)
Age (yr; mean $\pm$ SD)	65.7 $\pm$ 4.52	65.2 $\pm$ 4.39	66.1 $\pm$ 4.60
BMI (mean $\pm$ SD)	24.6 $\pm$ 3.36	23.9 $\pm$ 3.52	25.1 $\pm$ 3.14
SBP (mmHg; mean $\pm$ SD)	133.7 $\pm$ 19.2	134.7 $\pm$ 18.4	132.9 $\pm$ 19.8
DBP (mmHg; mean $\pm$ SD)	94.1 $\pm$ 30.8	95.9 $\pm$ 30.5	92.7 $\pm$ 31.1
Use of BP-lowering drugs ^c %	56.4	60.9	52.8
Glucose (mmol/l; mean $\pm$ SD)	5.29 $\pm$ 1.33	5.22 $\pm$ 1.10	5.35 $\pm$ 1.49
Use of glucose-lowering drugs ^c %	16.7	11.6	20.7
Cholesterol (mmol/l; mean $\pm$ SD)	5.30 $\pm$ 0.99	4.98 $\pm$ 0.84	5.54 $\pm$ 1.03
Serum creatinine (mol/l; mean $\pm$ SD)	76.8 $\pm$ 12.9	85.5 $\pm$ 12.7	69.9 $\pm$ 8.33
Activity ^b %	62.8	49.3	67.8
Smoking ^c %	58.3	58.0	60.9

^aData are means $\pm$ SD, BP: blood pressure; DBP: diastolic BP; SBP: systolic BP.

^bExercise more than twice 1 week.

POPULATION

Two hundred and two elderly citizens (≥ 60 years old) from Junliangcheng community, Dongli district, Tianjin, China were randomly selected. First and second morning urine were collected from each participants, successfully. Healthy characteristic information of participants was collected by clinician inquiry. Urine samples with occult blood determined by urine dipsticks were excluded. Finally, 191 participants were included, 44.5% male. Overall, the proportion of diabetes and hypertension were 16.8% and 56.0%, respectively, and 39.3% without diabetes and hypertension. The detail characteristics of the study population is listed in Table 1.

METHODS AND MATERIALS

We provided 10 ml urine tubes to 400 elderly people before the day elderly prior outpatient visit. The participants brought their first morning urine to the hospital in the morning, then they were asked to collect the second one during elderly prior outpatient. Finally, 191 participants were included. The elderly prior outpatient was from 7 to 9 o'clock every morning. We collected urine samples in 1.5 ml Eppendorf tubes, than took the samples to laboratory and stored at -80°C immediately. Before test, samples were allowed to return to room temperature, mixed totally by inversion, then centrifuged at $400 \times g$ for 10 min to remove the precipitation. We measured the urinary albumin concentration (UAC) and the urinary creatinine concentration, calculated ACR values, then we analyzed the correlation between second and first morning urine ACR. According to kidney disease outcomes global improving (KDIGO) (13), $\text{ACR} \geq 30 \text{ mg/g}$ was used as the cut-off value to assess albuminuria.

Urine analysis was performed on Di-Rui H800 urine analyzer (Changchun, China), urine albumin was de-

termined by immunonephelometry with Dade-Behring BNII special protein analyzer and manufacturer provided reagents. The lower detection limit of urine albumin was 2.02 mg/L. The intra-assay coefficient of variation (CV) was 3.0% at 12.5 mg/l and 2.1% at 165 mg/l. The determination of urine creatinine was performed on Abbott IC16000 and manufacturer provided reagents, the intra-assay CV for this assay was 3.4% at $340 \mu\text{mol/l}$. In our study the values of urine albumin which were lower than 2.02 mg/l were treated as 2.02 mg/l.

STATISTICAL ANALYSIS

Skewed distribution data were expressed as median and interquartile range, normal distribution as mean $\pm$ SD. Wilcoxon rank test and Bland–Altman plot were applied to compare the difference of values between ACR1 and ACR2 due to the skewed data for ACR. We used scatter-plots and calculated correlation index “r” to investigate the correlation between ACR1 and ACR2. Linear regression was used to calculate the slope and intercept (regarded the values of ACR1 as independent variable X), with log-transformed data. Consistency of urine albumin category by ACR1 and ACR2 was analyzed with Chi-square test. The concordance of estimating albuminuria (yes or not) between ACR1 and ACR2 was estimated by Kappa coefficient. We used SPSS 19 and Medcalc software to perform statistical analysis.

RESULTS

The test results of ACR1, ACR2 are displayed in Table 2. The median of ACR1 was slightly higher than ACR2 (17.55 vs. 16.47 for ACR). There was no statistical difference between ACR1 and ACR2 ($P = 0.271$).

TABLE 2. Means and Distributions Of ACR In Two Samples

	ACR1 (mg/g)	ACR2 (mg/g)
Means	31.84	34.37
5th percentile	5.19	6.01
25th percentile	9.35	9.63
50th percentile	16.47	17.55
75th percentile	31.67	34.78
95th percentile	119.44	120.17

ACR 1 is ACR in first morning urine samples; ACR 2 is ACR in second morning urine samples. The statistical difference between medians of two samples is analyzed by Wilcoxon rank test, $P = 0.271$ between ACR1 and ACR2.

TABLE 3. Correlations Between First Morning Voids and Second Morning Samples^a

	ACR (ACR1 vs. ACR2)	<i>P</i>
Overall ($n = 191$)	$r = 0.901$	<0.001
Men ($n = 85$)	$r = 0.938$	<0.001
Women ($n = 106$)	$r = 0.767$	<0.001
Diabetes ($n = 32$)	$r = 0.723$	<0.001
No diabetes ($n = 159$)	$r = 0.904$	<0.001
Hypertension ($n = 107$)	$r = 0.904$	<0.001
Non-hypertension ($n = 84$)	$r = 0.834$	<0.001
Diabetes with hypertension ($n = 23$)	$r = 0.771$	<0.001
Nondiabetes and Nonhypertension ($n = 75$)	$r = 0.850$	<0.001

^aData are analyzed using Spearman correlation method.

The correlation of ACR1 and ACR2 are displayed in Table 3. Overall, a good correlation was found between ACR1 and ACR2 ($r = 0.901$, $R^2 = 0.812$). The highest correlation was observed in male and the lowest was diabetes population. The slope and intercept of $\log(\text{ACR})$ were 0.93 and 0.11.

Figure 1 showed the Bland–Altman plot of ACR1 and ACR2 ($\text{ACR} < 300 \text{ mg/g}$). The dotted lines around zero line indicated the ± 1.96 standard deviation (SD) of the difference between ACR1 and ACR2. The larger the average of ACR1 and ACR2, the greater the difference of these values from zero level. There is more than 95% of difference in the range of ± 1.96 SD. However, the wide ranges of $1.96 \pm \text{SD}$ suggested that obvious bias between ACR1 and ACR2 occurred in many participants, especially in those whose ACR was higher than 50 mg/g.

Table 4 presents the prevalence of albuminuria in the participants. The percent of participants with increased ACR1 was 26.2%, participants with increased ACR2 was 28.3%. Totally, 23.6% of participants had increased ACR1 and ACR2 concomitantly. The prevalence of albuminuria evaluated by ACR2 was slightly higher than ACR1, but there was no statistical difference between them ($P = 0.730$). The performance of ACR1 and ACR2 to detect al-

buminuria was nearly the same in male and hypertension groups.

The concordance of assessing albuminuria between ACR1 and ACR2 (Kappa coefficient (κ)) is presented in Table 4. Overall, there was a good agreement for albuminuria status between ACR1 and ACR2 ($\kappa = 0.815$, 92.7%). The best agreement was observed in male group ($\kappa = 0.900$, 96.5%) and the lowest agreement ($\kappa = 0.724$, 87.5%) occurred in diabetes group relatively.

DISCUSSION

Albuminuria is an early biomarker to screen renal injury and plays an important role in the progression monitor of diabetic nephropathy and cardiovascular disease. Recently, numerous population-based studies had demonstrated that the elderly population showed a high prevalence of albuminuria. For example, the prevalence of albuminuria was 10.9% in a Spanish elderly population (4), 21.4% in a Chinese elderly population (5). In this study, the percent of participants with albuminuria was 23.6%, and the prevalence of albuminuria was higher in diabetes (28.1%) and hypertension group (29.0%). It was relevant to screen and monitor albuminuria in elderly people.

Many researchers have carried out studies that looked at which urine sample (a first morning void or random urine sample) and which urine albumin measurement (ACR or UAC) was the best surrogate to the 24 hr urine albumin excretion. It was reported that the lowest urine albumin intraindividual CV occurred in ACR from a first morning urine sample (14). Many studies recommended ACR from first morning voids as an alternative to 24 hr urine albumin excretion. However, for outpatients, it could be difficult to collect the first morning urine. A second morning void would be more practical.

Our study showed that ACR 2 was slightly higher than ACR 1, although no statistical difference was found (Wilcoxon rank test, $P = 0.271$). ACR indicated a low intraindividual CV between a first and second morning urine sample. ACR1 and ACR2 showed a good correlation, especially in male and hypertension group. On the basis of linear regression models of ACR 1 and ACR 2, the R^2 value was 0.812, indicating that 81.2% of the variation observed in ACR 2 can be explained by ACR 1. Figure 1 showed large bias between urine samples in many participants, especially in those with ACR higher than 50 mg/g.

In the urine albumin category, totally, there was a high concordance of assessing albuminuria between ACR 1 and ACR 2 ($\kappa = 0.815$, 92.7%), with a higher agreement in men, hypertension and nondiabetes groups. These findings were similar to the study by Saydah et al. (15), but the specific reasons were still unclear. On the other hand,

Fig. 1. Bland–Altman plot of ACR1 and ACR2 for participants with ACR <300 mg/g ($n = 189$).

we found that the correlations between ACR 1 and ACR 2 in diabetes patients as well as patients with diabetes and hypertension were low relatively. Maybe the excretion of albumin of those patients was larger.

It was regretful that our study did not include a random spot urine. Saydah et al. (15) had compared ACR in first morning urine samples to random urine collections in adult population, it indicated that the concordance of ACR category (increased or not) in the two samples was moderate in overall population ($\kappa = 0.51$). And according to our research, the concordance of ACR category between second morning voids and first morning urine samples was much higher ($\kappa = 0.815$). And that provided a side support: ACR from a second morning void was

more reliable than that from a random spot urine sample when compared albumin category results with a first morning void.

Above all, our results indicated that (1) there was a high prevalence of albuminuria among Chinese elderly population. (2) ACR from a second morning urine was highly consistent with that from a first morning urine when assessing albuminuria in Chinese elderly population. In addition, a second morning void would be more feasible to collect than a first morning urine void for outpatients. So we suggest that those patients with ACR less than 50 mg/g can be examined in the future with a second void, however, those patients with ACR more than 50 mg/g keep delivering a first morning void, despite the difficulties.

TABLE 4. Concordance between the two samples for albuminuria^a

	Increased first morning ACR ^b %	Increased second morning ACR ^b %	Increased both ACR1 and ACR2 ^c %	Concordance between the two samples	
				Percent agreement ^c %	κ
Overall ($n = 191$)	26.2	28.3	23.6	92.7	0.815
Men ($n = 85$)	22.4	23.5	21.2	96.5	0.900
Women ($n = 106$)	29.2	32.1	25.5	89.6	0.756
Diabetes ($n = 32$)	31.3	37.5	28.1	87.5	0.724
Nondiabetes ($n = 159$)	25.2	26.4	22.6	93.7	0.836
Hypertension ($n = 107$)	31.8	32.7	29.0	93.5	0.850
Nonhypertension ($n = 84$)	19.0	22.6	16.7	91.7	0.748
Diabetes with hypertension ($n = 23$)	34.8	34.8	30.4	91.3	0.808
Nondiabetes and nonhypertension ($n = 75$)	18.7	20.0	16.0	93.3	0.786

^aData are %. Increased ACR defined as ≥ 30 mg/g.

^bNo statistic difference between the prevalence of albuminuria estimated by ACR1 and ACR2 by Chi-square test, $P > 0.05$ in every group.

^cPercent agreement is defined as the value: [concordant number on diagonal / total] $\times 100\%$.

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CONFLICT OF INTEREST

No authors declared any potential conflicts of interest.

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