Screening for Microalbuminuria in Type 2 Diabetes : A Reconsideration

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Abstract

To study the effect of timing of urine collection in determination of microalbuminuria in type 2 diabetic patients, timed urine (night time and daytime) as well as spot urine (first morning and random morning) samples were collected from 44 type 2 diabetic patients, 21 with normoalbuminuria and 23 with microalbuminuria. The methods of spot urine albumin expression for microalbuminuria were also compared between albumin concentration (AC) and albumin to creatinine ratio (ACR). Night time albumin excretion rate (AER) was 16 per cent lower and daytime AER was 13 per cent higher than 24-h AER ($p<0.001$). Forty-one (93%) of both night time and daytime urine samples had results corresponding with 24-h AER. For the spot urine, expression as AC showed a slightly stronger correlation with 24-h AER than expression as ACR. The levels of albumin in random morning urine samples were 50 and 35 per cent significantly higher than those in first morning urine samples when expressed as AC and ACR, respectively. In conclusion, because of low diurnal variation of AER, either daytime or night time urine could be used for screening of microalbuminuria in type 2 diabetes. Since mean albumin levels obtained from random morning urine were higher than those obtained from first morning urine, the cut-off level should be set higher in random morning urine in order to give comparable sensitivity in predicting microalbuminuria. The spot urine, either first morning or random morning urine, had a good correlation with the 24-h AER whether expressed as AC or ACR. Given the cost of the latter, the authors suggested measuring spot urine AC instead of ACR for screening of microalbuminuria in type 2 diabetes.

Key word : Microalbuminuria, Type 2 Diabetes, Screening

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The American Diabetes Association (ADA) has recently recommended that screening for microalbuminuria can be performed by three methods: 1) measurement of the albumin to creatinine ratio (ACR) in a random spot collection; 2) 24-h collection with creatinine; and 3) timed collection[1]. For timed collection, the microalbuminuric range is defined by albumin levels of 20-200 μg/min regardless of the urine collection time. For random spot collection, first-void or other morning collections are preferred and the same cut-off value is used for the determination of microalbuminuria. In the authors' opinion, some aspects of the recommendation should be worth reconsidering. Firstly, as there is a circadian rhythm of albumin excretion rate (AER), the time of urine collection should be considered when defining the microalbuminuric range. Previous studies, mostly in type 1 diabetics, showed that AER from the daytime collections were significantly higher than those obtained from the night time[2-8]. Secondly, such physiological factors as upright posture and exercise can affect AER[9,10], therefore, the first morning urine sample should be preferred rather than the other random morning urine sample. Thirdly, the merit of using ACR instead of albumin concentration (AC) in the detection of microalbuminuria in type 2 diabetes is questionable. In type 1 diabetes, the urine flow rate may vary markedly depending on the degree of glycemia, therefore spot urine samples expressed as ACR are probably better indicators of the disease than those expressed as AC. However, given that glycemia of most type 2 diabetic patients is relatively stable, the adjustment of AC by urine creatinine concentration may have no further advantage.

ADA recommendations for screening microalbuminuria is based on studies mostly taken from type 1 diabetic patients. Although these recommendations can conceivably be applied to type 2 diabetic patients the data directly taken from type 2 diabetic patients should be more appropriate. This study was undertaken to compare timed urine specimens (night time, daytime) and spot urine specimens (first morning and random morning) as well as albumin expression (AC and ACR) in determining microalbuminuria in type 2 diabetic patients.

MATERIAL AND METHOD

Oral hypoglycemic agent-treated type 2 diabetic patients without overt nephropathy (AER of more than 200 μg/min) were enrolled from the outpatient diabetic clinic at Songklanagarind Hospital. Forty-four type 2 diabetic patients consisting of 20 women and 24 men were included in this study. Their mean age was 55.39±11.00 (SD) years (range 32-85) with body mass index of 25.60±3.92 kg/m^2 (range 16.44-36.41). The duration of diabetes was 9.06± 5.32 years (range 2-25). After obtaining informed consent, patients received written and verbal instructions on how to collect urine. No specific recommendation was made about physical exercise or intake of dietary protein and fluid. Patients were instructed to collect 24-h urine samples in three portions one day before his or her clinic visit. Portion 1 was composed of all urine collected after passing the first morning urine voiding until the last urine was collected immediately before bed time. Portion 2 was composed of all urine collected during the night. Portion 3 was the first morning urine sample. On the day of the clinic visit, patients were asked to void at the outpatient clinic. This specimen was designated random morning urine sample. The time of each urine collection was also recorded on record forms at the first voiding, last voiding before going to bed and the first morning voiding. The urine from portion 1 was designated daytime. The urine from the combination of portions 2 and 3 was designated night time. The combination of daytime and night time urine was considered the 24-h urine. Urine creatinine was also measured in the 24-h urine samples to test for the adequacy of collection which would be considered satisfactory if they contained 10 mg of creatinine/kg ideal body weight for females or 15 mg of creatinine/kg ideal body weight for males. After measuring the volume, an aliquot of each portion of urine sample was stored at 4°C for analyses of albumin and creatinine within one week of collection.

Urine albumin was measured by the immunoturbidimetric method using a Hitachi 704 analyzer (Boehringer Mannheim, Mannhiem, Germany). The intra-and interassay coefficient of variations were 2.40 and 2.38 per cent, respectively. Urine creatinine was measured by modified Jaffé method. AER was expressed as micrograms per minute for timed urine. AC was determined as milligrams per liter and ACR as milligrams per gram of creatinine for spot urine.

Statistical analysis

All urinary albumin results were log10 transformed before analysis because of the positively skewed distribution. After log10 transformation, the

data showed homogeneity of variance. Results are shown as geometric mean (antilog SD). Comparison between two groups was performed by using the Student's *t*-test. The relationship between 24-h AER and AC or ACR was determined by Pearson's correlation coefficient. Stata version 6 was used for computations. $p<0.05$ was considered significant.

The performance of measurement of spot urine AC and ACR was assessed by using the receiver operating characteristic (ROC) curve approach. The nearest point to the intersection of the ROC curve with 100 per cent-to-100 per cent diagonal which represented the best equilibrium between sensitivity and specificity was chosen as the cut-off value. Twenty-four hour urine AER was used as the gold standard, levels below 20 μg/min were considered normal, whereas, levels between 20-200 μg/min were considered microalbuminuria.

RESULTS

Samples were divided into two groups according to 24-h AER: normoalbuminuria (AER< 20 μg/min: n = 21) and microalbuminuria (AER 20-200 μg/min: n = 23). Table 1 shows AER of night time, daytime and 24-h urine. The AER of night time was 23 per cent lower than that of daytime and 16 per cent lower than the AER of the 24-h urine while the AER of daytime was 13 per cent higher than that of the 24-h urine. The magnitude of difference was greater in microalbuminuric than in normoalbuminuric ranges (data not shown). Forty-one (93%) of both night time and daytime urine AER had results corresponding with 24-h AER.

The correlation of 24-h AER and spot urine samples expressed as AC and ACR is shown in Fig. 1. Of both the first morning and random morning urine samples, those expressed as AC showed a slightly stronger correlation with 24-h AER than those expressed as ACR. The levels of albumin in random morning urine samples were 50 and 35 per cent, significantly higher than those in the first morning urine sample when expressed as AC and ACR, respectively ($p<0.001$) (Table 2). When 30-300 mg/g creatinine was classified as a microalbuminuric range value according to ADA recommendation, 35 (80%) and 31 (70%) of the results obtained from the first morning and random urine specimens, respectively agreed with AER of the 24-h urine.

Fig. 2 depicts the ROC curves for AC and ACR of the first morning and random morning urine samples in the determination of microalbuminuria using the 24-h AER as a reference method. The areas under the ROC curve for the first morning urine were 0.9441 for AC and 0.9337 for ACR. The corresponding areas for random morning urine were 0.9410 and 0.9317, respectively. The cut-off points for the first morning urine specimens, according to the nearest point to the intersection of the curves with 100 per cent-to-100 per cent diagonal, were 18.7 mg/l and 27.4 mg/g creatinine for AC and ACR, respectively. The corresponding values for random morning urine specimens were 34.1 mg/l and 41.2 mg/g creatinine. The sensitivity and specificity for the given cut-off points are shown in Table 2.

DISCUSSION

Several studies reported the AER of night time AER to be significantly lower than that of daytime in type 1 diabetic patients with normo- and microalbuminuria[2-8]. In contrast, only a relatively few studies compared night time and daytime AER in type 2 diabetes. Mogensen et al reported that AER of the night time was 36 per cent and 25 per cent lower than that of the daytime and the 24-h urine respectively in type 1 diabetic patients[2,4]. In contrast to Mogensen's study, the present study showed a respective difference of only 23 per cent and 16 per cent among type 2 diabetic patients. The

Table 1. Geometric mean (antilog SD) of AER in night time, daytime and 24-h urine specimens.

	Night time AER	Daytime AER	24-h AER
Normoalbuminuria (n=21)	5.90 (1.96)*	8.32 (1.93)	7.02 (2.08)†
Microalbuminuria (n=23)	38.81 (2.00)*	56.53 (1.87)	52.64 (1.83)†
All (n=44)	15.80 (3.21)*	22.65 (3.18)	20.12 (3.36)†

* $p < 0.001$ comparing night time AER with daytime and 24-h AER
† $p < 0.001$ comparing daytime AER with 24-h AER
AER = albumin excretion rate

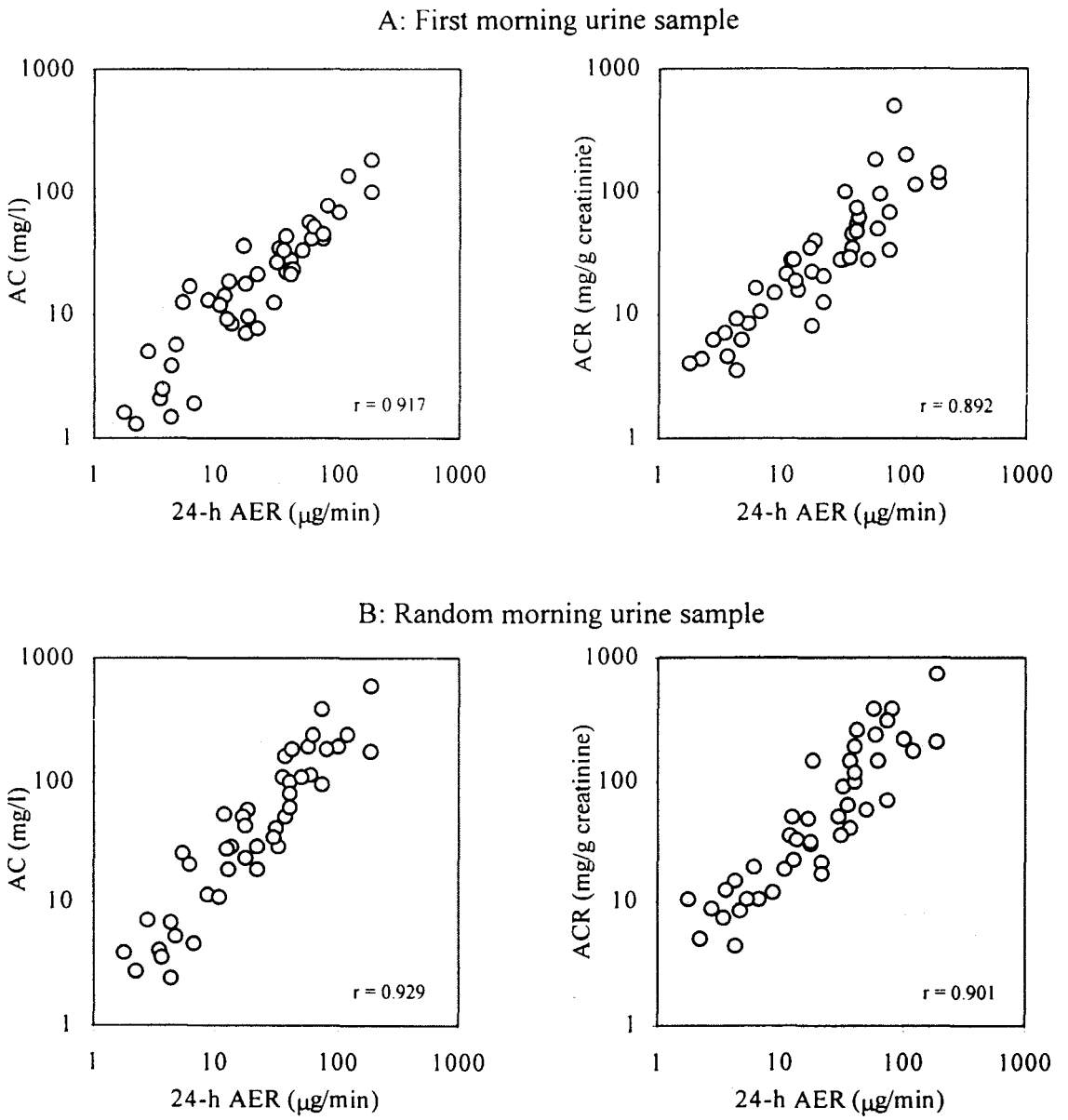

Fig. 1. Relationship between 24-h AER and the first morning urine (A) and random morning urine (B) expressed as AC (left) and ACR (right) (results are plotted on log-log scale).

present findings correspond to those of Tomaselli et al[5] who also demonstrated a lesser degree of diurnal variation of AER in type 2 diabetes. Since the lesser degree of diurnal variation of AER in type 2 diabetic patients in the present study, the urine AER whether obtained from the daytime or night time urine could be used to correctly classify the albumin status in most (93%) of the presented patients. On the other hand, Stehouwer et al[8] reported in type 1 diabetic patients that only 21 of 28 (75%) of the timed urine samples were correctly classified when night time urine samples were used.

Table 2. Geometric mean, cut-off points, sensitivity and specificity of AC and ACR of spot urine as a screening test for microalbuminuria.

	First morning urine		Random morning urine	
	AC	ACR	AC	ACR
Geometric mean (antilog SD)	16.25 (3.40)	27.40 (3.16)	37.57 (4.24)	47.59 (3.75)
Cut-off point	18.7	27.4	34.1	41.2
Sensitivity (%)	91.3	86.9	86.9	86.9
Specificity (%)	90.5	80.9	80.9	85.7

AC = albumin concentration; ACR = albumin to creatinine ratio

Among type 2 diabetic patients, those with normoalbuminuria were shown to have a significantly higher daytime AER than night time AER[5,7], but the difference between day and night was lost in patients with microalbuminuria[7]. Surprisingly, the present study has demonstrated a greater diurnal variation of AER in patients with microalbuminuria than those with normoalbuminuria. Why these results differ from the others is not known. However, there are possibly several factors that could affect the magnitude of diurnal variation of AER, such as, the degree of albuminuria, diurnal variation of blood pressure or autonomic neuropathy. Spallone et al[11] found that the mean blood pressure values and mean 24-h AER did not significantly differ between type 1 diabetes with and without autonomic neuropathy while the percentages of day-night changes in blood pressure and AER were significantly lower in those with autonomic neuropathy. The association between day-night changes in blood pressure and AER in type 2 diabetic patients needs further study.

Whereas ACR is recommended in the screening of microalbuminuria instead of AC as it is independent of the urine flow rate, the authors found no advantage in using ACR compared with AC for estimating the 24-h AER. Although Bakker et al and Hutchison et al independently demonstrated that the measurement of ACR showed a stronger correlation with AER of the timed urine than did the measurement of AC[12,13], however, they measured both AC and ACR from the same specimens of the timed urine samples which could not be extrapolated to the spot urine samples. If the spot urine sample, which is more practical, was collected separately as conducted in the present study, the correlation of AC and ACR with AER may be different. In addition, the previous studies[2,3] did not specify the type of diabetes which theoretically can affect the AC results. Since excessive glycemic excursion in type 1 diabetes may cause the urine flow rate to fluctuate markedly, using AC may cause an overestimate of AER in concentrated urine and an underestimate of AER in diluted urine. This problem can be overcome by correcting the urine creatinine concentration. In contrast to type 1 diabetes, glycemia of most type 2 diabetic patients is relatively stable, therefore, the adjustment of AC by using urine creatinine concentration would not be beneficial. This notion was supported by a recent study undertaken by Zelmanovitz et al in which 95 type 2 diabetes were evaluated [14]. They found that AC and ACR, measured in random spot urine samples, showed a similar accuracy for screening microalbuminuria.

Although the correlation of AC and ACR in either the first morning or random morning urine was comparable to the 24-h AER, the mean values obtained from random morning urine were 30-50 per cent higher than those obtained from the first morning urine. This difference has been ascribed to the effects of upright posture and exercise on albumin excretion[9,10]. If the same cut-off level for detecting microalbuminuria was used, the results obtained from the first morning urine would be expected to have a lower sensitivity and higher specificity than those obtained from the random morning urine. This phenomenon also occurred in type 1 diabetes[15]. In the present study, the cut-off level that represented the best equilibrium between sensitivity and specificity was 27.4 mg/g creatinine of the first morning urine, similar to 30 mg/g creatinine as recommended by ADA[1]. If the random morning urine was used, the corresponding level would be higher at 41.2 mg/g creatinine to give the same sensitivity (86.9%).

In conclusion, with the urine AER of 20 μg/min as a cut-off level of microalbuminuria in the timed urine, either daytime or night time, could be used for the screening of microalbuminuria in type

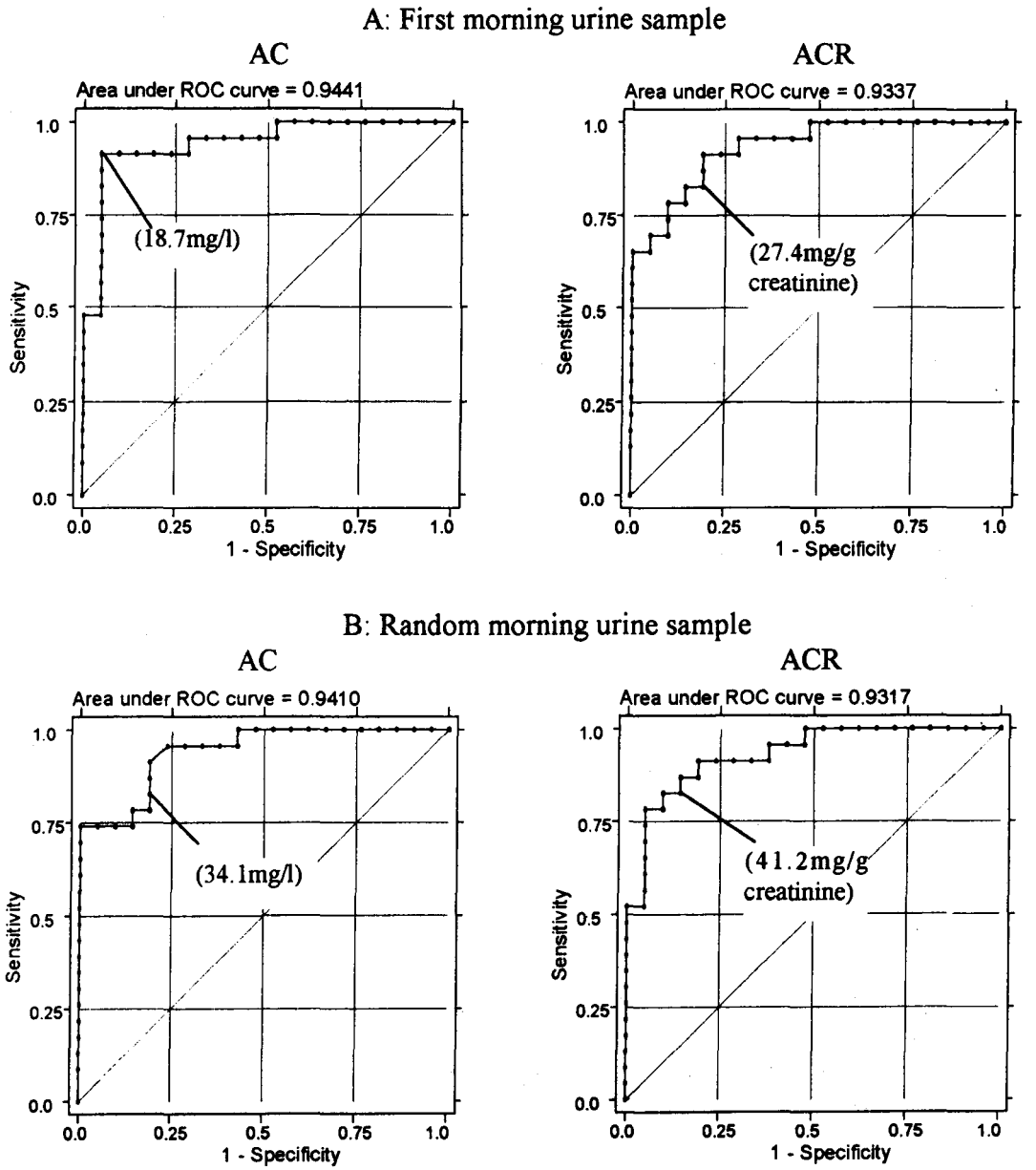

Fig. 2. ROC curves for albumin measurement (AC and ACR) in the first morning and random morning urine samples as a screening test for microalbuminuria. Values in parentheses correspond to the nearest point to the intersection of the curve with 100 per cent-to-100 per cent diagonal.

2 diabetes although the night time AER was found to be lower than daytime AER. Since the mean values obtained from the random morning urine were 30-50 per cent higher than those from the first morning urine, such a cut-off level should be set to a higher level in the random morning urine than in the first morning urine in order to give comparable sensitivity in predicting microalbuminuria. The spot urine, either the first morning or random morning urine, had a good correlation with the 24-h AER whether

expressed as AC or ACR. Given the cost of the latter, the authors suggest measuring spot urine AC instead of ACR for the screening of microalbuminuria in type 2 diabetes.

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การตรวจคัดกรองไมโครอัลบูมินในปัสสาวะในผู้ป่วยเบาหวานชนิดที่ 2 : ข้อควรพิจารณาอีกครั้ง

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เปรียบเทียบปริมาณอัลบูมินในปัสสาวะที่เก็บเป็นช่วงเวลา (timed urine) คือ ช่วงกลางคืน (night time urine) ช่วงกลางวัน (daytime urine) และปัสสาวะที่เก็บครั้งเดียว (spot urine) คือ ครั้งแรกหลังตื่นนอน (first morning urine) และที่เก็บแบบสุ่มในเวลากลางวัน (random morning urine) ของผู้ป่วยเบาหวานชนิดที่ 2 จำนวน 44 คน 21 คนมีระดับอัลบูมินในปัสสาวะอยู่ในเกณฑ์ปกติ (normoalbuminuria) และ 23 คนมีไมโครอัลบูมินในปัสสาวะ (microalbuminuria) และเปรียบเทียบ albumin concentration (AC) กับ albumin to creatinine ratio (ACR) ในปัสสาวะที่เก็บครั้งเดียว

ผลการศึกษาพบว่า albumin excretion rate (AER) ของปัสสาวะช่วงกลางคืนน้อยกว่า AER ของปัสสาวะ 24 ชั่วโมง (24-hour urine) ร้อยละ 16 ขณะที่ AER ของปัสสาวะช่วงกลางวันมากกว่า AER ของปัสสาวะ 24 ชั่วโมงร้อยละ 13 ($p<0.001$) ร้อยละ 93 ของปัสสาวะทั้งช่วงกลางวันและช่วงกลางคืนมีผลไปในทางเดียวกับปัสสาวะ 24 ชั่วโมง สำหรับปัสสาวะที่เก็บครั้งเดียว AC มีความสัมพันธ์กับ AER ของปัสสาวะ 24 ชั่วโมงมากกว่า ACR เล็กน้อย ระดับอัลบูมินในปัสสาวะที่เก็บแบบสุ่มในเวลากลางวันมากกว่าในปัสสาวะครั้งแรกหลังตื่นนอนร้อยละ 50 (AC) และ 35 (ACR)

สรุป เนื่องจาก diurnal variation ของ AER น้อย จึงสามารถใช้ปัสสาวะช่วงกลางวันหรือกลางคืนในการตรวจคัดกรองไมโครอัลบูมินในปัสสาวะในผู้ป่วยเบาหวานชนิดที่ 2 ก็ได้ ระดับอัลบูมินในปัสสาวะที่เก็บแบบสุ่มในเวลากลางวันมากกว่าในปัสสาวะครั้งแรกหลังตื่นนอน ดังนั้นควรกำหนดเกณฑ์ของไมโครอัลบูมินในปัสสาวะที่เก็บแบบสุ่มในเวลากลางวันให้สูงขึ้นเพื่อให้ความไว (sensitivity) ใกล้เคียงกัน ระดับอัลบูมินในปัสสาวะที่เก็บครั้งเดียว ไม่ว่าเป็น AC หรือ ACR ก็มีความสัมพันธ์กับ AER ของปัสสาวะ 24 ชั่วโมง ดังนั้นในแง่ของค่าใช้จ่าย จึงแนะนำให้ใช้ AC แทน ACR ในการตรวจคัดกรองไมโครอัลบูมินในปัสสาวะในผู้ป่วยเบาหวานชนิดที่ 2

คำสำคัญ : ไมโครอัลบูมินในปัสสาวะ, เบาหวานชนิดที่ 2, การตรวจคัดกรอง

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