Why 24-h Urine Albumin Excretion Rate Method Still is Used for Screening of Diabetic Nephropathy in Isfahan Laboratories?

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Date of Submission: Jul 09, 2013

Date of Acceptance: Dec 16, 2013

How to cite this article: Teimoury A, Iraj B, Heidari-Beni M, Amini M, Hosseiny S. Why 24-h urine albumin excretion rate method still is used for screening of diabetic nephropathy in Isfahan laboratories?. Int J Prev Med 2014;5:341-7.

ABSTRACT

Background: The first step in diagnosis of diabetic nephropathy is measurement of albumin in a spot urine sample. The aim of this study was assessment of the accuracy of urinary albumin to creatinine ratio (UACR) in random urine specimens (RUS) for microalbuminuria and macroalbuminuria screening in Iranian diabetic patients.

Methods: A total of 200 diabetic patients participated to our study. 24 h timed urine specimens followed by RUS were collected. 24-h urine albumin excretion (24-h urinary albumin excretion (UAE)) and UACR in RUS were measured. Data were analyzed by Pearson's correlation, receiver operating characteristic (ROC) curve and McNemar test.

Results: A total of 165 patients finalized the study. Pearson's correlation of coefficient for 24-h UAE versus UACR was 0.64. The area under ROC curve for UACR was 0.83 in microalbuminuria and 0.91 in macroalbuminuria. The cutoff point of 30 mg/g in UACR method had 86% sensitivity and 60% specificity for microalbuminuria screening and cut-off point of 300 mg/g had 75% sensitivity and 99% specificity for macroalbuminuria screening respectively.

Conclusions: UACR in RUS showed acceptable performance as a screening test for diagnosis of both micro and macroalbuminuria in Iranian diabetic patients.

Keywords: Albuminuria, diabetic nephropathy, screening, urinary albumin to creatinine ratio

Original Article

INTRODUCTION

Diabetic nephropathy is one of the microvascular complications of diabetes that occurs in 20-40% of diabetic patients.[1] Prevalence of diabetic nephropathy is varied in different races and has high prevalence among Asian races.[2] Patients with uncontrolled blood sugar, blood pressure and lipid profiles; are at greater risk of diabetic nephropathy.[3] Study among type II diabetic Chinese patients showed that approximately 8% of diabetic patients had co-existing diabetic nephropathy and non-diabetic renal disease.[4]

Microalbuminuria is usually the first sign of diabetic nephropathy. Furthermore microalbuminuria is one of the major risk factors of cardiovascular disease (CVD) and CVD-related death especially in type II diabetes.[5,6] Diabetic nephropathy progresses from microalbuminuria to macroalbuminuria, renal failure and end stage of renal disease. Thus, early diagnosis of microalbuminuria by screening tests and early treatment is very important to prevent kidney disease progression, CVD events and CVD-related death.[7,8]

Various screening methods of albuminuria were recommended by American Diabetes Association. There are three methods of urine albumin measurement to screen and diagnosis of diabetic nephropathy and albuminuria. These methods are 24-h urine collections, timed urine collections and random urine specimens (RUS) that in third method urinary albumin to creatinine ratio (UACR) (mg/g or mg/mmol) is calculated.[9,10] At 24-h urine collection for urine albumin measurement is gold standard method to microalbuminuria screening.[11] However, this method is laborious, inconvenient and susceptible to errors related to collecting samples. UACR in RUS is cheaper and easier than 24-h urine collection method. Some studies investigated and compared the diagnostic value of these methods and suggested albuminuria cut-off values.[12-14] Since microalbuminuria could be population dependent, the best and easiest methods and cut-off point for diagnosis of microalbuminuria should be detected in each country and races. Despite measurement of UACR is easier than urinary albumin excretion (UAE), but laboratories still use UAE collection method for collecting urine sample of patients in Isfahan. The present study was designed to investigate accuracy of UACR in RUS in diabetic patients in Isfahan city compare with 24-h UAE. We want to inform laboratories in Isfahan that UACR in RUS is preferred to UAE method and laboratories can trust UACR method for diagnosis of micro and macroalbuminuria.

METHODS

Participant

This cross-sectional study was done on 200 diabetic patients referred to Isfahan Endocrine and Metabolism Research Center (IEMRC). Patients were selected by systematic sampling. Type I and type II diabetic patients were included. Exclusion criteria were history of severe heart failure, kidney disease except diabetic nephropathy, urinary tract infections, hematuria (presence of 5 or more red blood cells in urine), positive urine culture and pyuria, abnormal urinary sediment, dehydration, fever and heavy physical activity during 24 h preceding the urine collection, pregnancy and 24-h urinary creatinine <700 mg/day and 1000 mg/day for women and men, respectively.[15,16] The IEMRC Medical Ethics Committee approved this study and each participant filled in consent.

Anthropometric and blood pressure assessment

Demographic questionnaire was completed for each patient. Height was measured in a standing position by meter to the nearest 1 cm without shoes. Weight was measured without shoes and with minimal clothing by Seca scale to the nearest 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Blood pressures were taken using a standardized mercury sphygmomanometer on the right arm, after a 5 min rest in a sitting position. Hypertension was defined as blood pressure more than 140/90 mmHg or patients treated with blood pressure-lowering drugs.[17]

Analytical methods

Patients were trained how properly collect urine and they were asked to provide a 24-h urine collection. We trained patients that a 24-h urine collection must be started at a specific time and then ended at the same time the next day. Patients used a clean glass container to catch their urine each time and put the urine into the collection container each time they urinate. The 24-h urine test began by urinating directly into the toilet. After urinating (empty the bladder) for the first time, patients noted the exact time and the urine collection began at this time. After delivery of 24-h urine specimens to IEMRC, 5cc blood sample was taken for measurement of fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c) and serum creatinine. Furthermore, one RUS was taken from each patient.

Albumin and creatinine measurements were performed on each urine collection (24-h urine specimens and RUS). Urinary albumin was

measured by auto analyzed (Liasys, Roma, Italy) and immunoturbidimetric method (Pars Azmun, Tehran, Iran) that Inter-assay coefficients of variations was 2.93% and intra-assay coefficients of variations was 1.31%. Urinary creatinine was determined by the Jaffe colorimetric assay and auto analyzed (Liasys, Roma, Italy) using commercial kits (Pars Azmun, Tehran, Iran). Intra-assay coefficient of variations was 3.22%.

HbA1c was determined by DS5 and Ion Exchange Chromatography. Blood glucose was measured by enzymatic method (GOD/PAP) and commercial kits (Pars Azmun, Tehran, Iran) and serum creatinine level was determined by Jaffe colorimetric assay.

Albuminuria according to 24-h UAE was divided into three groups: Normoalbuminuria (albumin < 30 mg/24 h), microalbuminuria (30 mg/24 h < albumin < 300 mg/24 h) and macroalbuminuria (albumin ≥ 300 mg/24 h).

Patients according to UACR in RUS were divided into three groups: Normoalbuminuria (albumin < 30 mg/g), microalbuminuria (30 mg/g < albumin < 300 mg/g) and macroalbuminuria (albumin ≥ 300 mg/g).[18]

Statistical analysis

All data were analyzed by SPSS Inc, Chicago, IL, USA (version 16) software. Continuous variables presented as mean ± standard deviation (SD). Categorical variables were analyzed by Chi-square test and McNemar test. Association between two methods of UAE and UACR was determined by Pearson correlation coefficient. Sensitivity and specificity of UACR method as screening test of microalbuminuria and macroalbuminuria were determined by receiver operating characteristic (ROC) analysis. P <0.05 was considered to be statistically significant.

RESULTS

Totally 200 diabetic patients were participated in our study. Of these, 35 patients were excluded due to incorrectly 24-h urine specimens (24-h urinary creatinine <700 mg/day and 1000 mg/day for women and men, respectively) were excluded. Finally 165 patients completed the study. 66 patients (40%) and 99 patients (60%) were male and female, respectively. 148 subjects (93.7%) had type II diabetes and 10 subjects (6.3%) had type I diabetes. Prevalence of hypertension was 49% respectively.

Mean ± SD of variables such as age, BMI, blood pressure, serum creatinine, FBS, HbA1c and duration of diabetes were shown in Table 1.

We found a significant and positive association between UACR and HbA1c, between 24-h urinary albumin and systolic blood pressure, between 24-h urinary albumin and duration of diabetes, between age and systolic and diastolic blood pressure, between BMI and systolic and diastolic blood pressure and between HbA1c and duration of diabetes.

Pearson's correlation between 24-h UAE and UACR was 64% (P < 0.0001). Concordance rate among categories of 24-h UAE and UACR in RUS was shown in Table 2. According to this table, normoalbuminuria was diagnosed in 10 urine samples of patients in both 24-h UAE and UACR, microalbuminuria was diagnosed in 9 urine sample of patients in both 24-h UAE and UACR and macroalbuminuria was diagnosed in 113 urine sample of patients in both 24-h UAE and UACR. Concordance rate among 24-h UAE and UACR were 50% in normoalbuminuria group, 85% in microalbuminuria group and 75% in macroalbuminuria group that there was not any significant difference between two methods of 24-h UAE and UACR according to McNemar test [Table 2].

Sensitivity and specificity of UACR method for diagnosis of microalbuminuria with cutoff point of 30 mg/g were 86% and 60% respectively and for diagnosis of macroalbuminuria with cut-off point of 300 mg/g were 75% and 99% respectively. The area under the ROC curve (AUC) for microalbuminuria and macroalbuminuria in

Table 1: Clinical and para-clinical characteristics of participant

Variables	Mean±SD
Age (year)	49±13
Duration of diabetes (year)	7.6±5.6
BMI (kg/m^2)	28.8±5.2
Systolic blood pressure (mmHg)	124.8±22.5
Diastolic blood pressure (mmHg)	79.3±12.5
Serum creatinine (mg/dl)	0.93±0.19
FBS (mg/dl)	130.5±43.9
HbA1c (%)	6.85±1.55

BMI=Body mass index, FBS=Fasting blood sugar, HbA1c=Glycosylated hemoglobin, SD=Standard deviation

UACR method were 0.83 (CI: 95% =0.74-0.92) and 0.91 (CI: 95% =0.8-1) respectively [Tables 3 and 4].

Cut-off point of urinary albumin to creatinine ratio in random urine specimens for screening of microalbuminuria and macroalbuminuria in receiver operating characteristic (ROC) curve were shown in Figures 1 and 2.

DISCUSSION

In this study, we compared UACR in RUS as screening test of microalbuminuria and macroalbuminuria with 24-h UAE as gold standard test. We found strong correlation between two methods (r = 0.64, P < 0.0001). Incerti *et al*.[16] in their study have reported 0.74 Pearson correlation coefficient (P < 0.0001) and confirmed the strong association between two methods. Our results were showed that there was not any difference between normoalbuminuria, microalbuminuria and macroalbuminuria groups in UACR and UAE methods. Ahn *et al*.[15] showed concordance rate between 24-h UAE and UACR in normoalbuminuria, microalbuminuria and macroalbuminuria group were 92%, 63% and 88% respectively that they did not find any differences between 24-h UAE and UACR methods to diagnosis of albuminuria

Our study, according to AUC for microalbuminuria and macroalbuminuria in UACR method confirmed UACR method has acceptable accuracy for diagnosis of albuminuria in diabetic patients. AUC for microalbuminuria in another studies were 0.82,[15] 0.92[16] and 0.94[19] that these results confirmed precision of UACR method for screening of microalbuminuria.

The cut-off point of 30 mg/g in UACR method had 86% sensitivity and 60% specificity for diagnosis of microalbuminuria when 24-h UAE was the reference standard. Reduction cutoff points

Table 2: Concordance rate between UACR and 24-h UAE methods

UAE	UACR			
	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	Total
Normoalbuminuria	10	10	0	20
Microalbuminuria	18	113	2	133
Macroalbuminuria	0	3	9	12
Total	28	125	11	165

UACR=Urinary albumin to creatinine ratio, UAE=Urine albumin excretion

Table 3: Sensitivity and specificity of UACR method for screening microalbuminuria in diabetic patients

Cut-off level (mg/g)	Total (%)		Men (%)		Women (%)	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
20.4	97	40	97	25	98	44
26.6	90	50	92	50	90	50
30	86	60	89	50	86	50
34.8	81	70	79	75	82	69
36	78.5	75	76	75	81	75

UACR=Urinary albumin to creatinine ratio

Table 4: Sensitivity and specificity of UACR method for screening macroalbuminuria in diabetic patients

Cut-off level (mg/g)	Total (%)		Men (%)		Women (%)	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
78	92	75	100	87	86	82
179	83	98	100	100	71	98
248.3	75	99	80	100	71	98
300	75	99	80	100	71	98

UACR=Urinary albumin to creatinine ratio

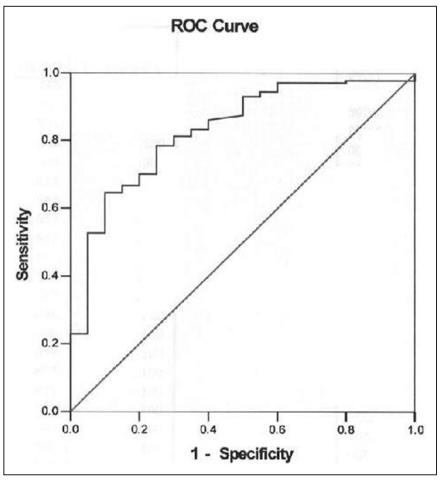

Figure 1: Cut-off point of urinary albumin to creatinine ratio in random urine specimens for screening of microalbuminuria in receiver operating characteristic curve

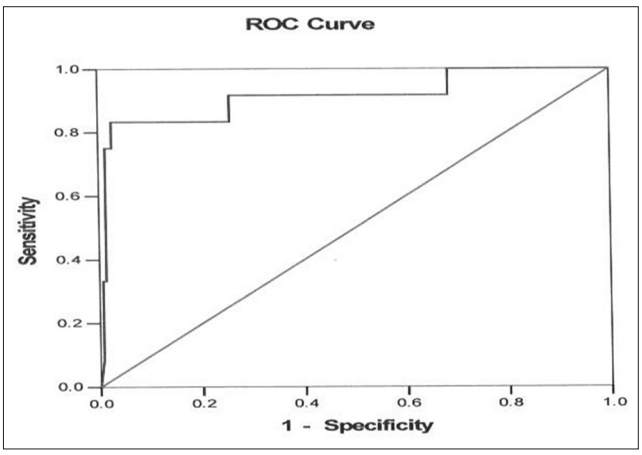

Figure 2: Cut-off point of urinary albumin to creatinine ratio in random urine specimens for screening of macroalbuminuria in receiver operating characteristic curve

from 30 mg/g to 26.6 or 20.4 mg/g lead to increase sensitivity and decrease specificity. To solve this problem, we recommended when UACR in RUS is more than 20 mg/g, 24-h UAE is performed to ensure the presence of microalbuminuria

Sensitivity and specificity in UACR method for macroalbuminuria were 75% and 99%, respectively in cut-off point of 300 mg/g. these findings showed UACR can be an acceptable method for screening of macroalbuminuria

Justesen *et al*.[20] studied UACR method on pregnant women with type I diabetes. They reported cut-off point of 30 mg/g had 83% sensitivity and 100% specificity for screening of microalbuminuria. Reduced cut-off point to 21 mg/g led to increase sensitivity to 100%. Furthermore they selected cut-off point of 210 mg/g for screening of macroalbuminuria.

Ahn *et al*.[15] showed 76.7% sensitivity and 92% specificity in cutoff point of 32.5 mg/g in UACR method for diagnosis of microalbuminuria. Incerti *et al*.[16] suggested cut-off point of 32 mg/g with 91% sensitivity and 92% specificity screened properly microalbuminuria.

Lambers Heerspink *et al*.[21] in their study have reported that UACR versus 24-h UAE was a good predictor for cardiovascular mortality and all-cause mortality. According to cross-sectional studies, good correlation was seen between UACR and 24-h UAE. Since 24-h UAE is inconvenience for patients, UACR is a good alternative to measuring 24-h UAE.[22,23] Hoefield *et al*.[24] consistently with our result suggested that AUC method could be used by clinicians for prediction renal function disorders in diabetic patients.

This study showed, replacing UACR method instead of 24-h UAE is reliable and affordable for screening of microalbuminuria and macroalbuminuria in Iranian diabetic patients. Most of the studies were performed in European countries and their results cannot be generalized to Middle Eastern countries like Iran. Our study showed UACR method is easier and more acceptable for Iranian diabetic patients. We suggest laboratories use UACR method for screening of microalbuminuria and macroalbuminuria and diagnosis of diabetic nephropathy.

Our study had some limitations. 24-h UAE and UACR in RUS were done one time and if each method was performed at least three times, more acceptable results obtain. Test would have more accuracy, if instead of RUS; the first urine in the morning was collected.

CONCLUSIONS

Our study supported UACR method for screening of microalbuminuria and macroalbuminuria and diagnosis of diabetic nephropathy in Iranian diabetic patients. UACR method is accurate and easy to perform. We suggest this method instead of 24-h UAE for screening of albuminuria in Iranian diabetic patients.

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Source of Support: Nil, **Conflict of Interest:** None declared.