



Study of Various Measures of Urine Albumin Excretion in 24-Hours Urine and Their Clinical Outcome in Type 2 Diabetic Patients

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Abstract

Background: Microalbuminuria (MAU) has been considered the first indication of renal injury in patients with diabetes and associated with a higher cardiovascular risk. It is evident from epidemiological studies that urine albumin concentration (UAC) or albumin: creatinine ratio (ACR) in spot urine sample gives similar result like 24-hour urinary albumin excretion (UAE).

Objectives: The study was aimed to find out whether UAC and/or ACR calculated in 24-hour urine sample correlate or not with same 24-hour UAE and what is diagnostic outcome by various measures of albumin excretion calculated in 24-hour urine samples in type 2 diabetic patients?

Methodology: We collected 24-hour urine sample from 125 type 2 diabetic patients and urinary albumin and creatinine were measured. We calculated different parameter of albumin excretion in 24-hour urine sample and interpretation of each parameter was derived. Data was expressed as median and range.

Results: The median ACR was 187.30 (mg/g), UAC was 135.5 (mg/L) and UAE was 270.92(mg/24 hours) in 24-hours urine sample. Clinical outcome of nephropathy by different calculated measures of albumin excretion in 24-hour sample was different. 72 patients had similar interpretation while 53 patients showed different diagnostic interpretation by different measures of albumin excretion in the same 24-hour urine sample.

Conclusion: Various measures of urine albumin excretion like UAC, ACR calculated in 24-hour urine sample in type 2 diabetic patients showed different diagnostic interpretation. We recommend when the urinary albumin concentration exceeds 10 mg/L in spot or random urine sample, another 24-hour sample should be collected for confirmed diagnosis of the stage of the albuminuria / nephropathy. We conclude that 24-hour urine sample is gold standard for diagnosis of nephropathy in diabetic patients. Further prospective studies are needed to confirm our findings.

Key Words: Microalbuminuria, Type 2 diabetes mellitus, Urine albumin concentration, albumin: creatinine ratio, Urinary albumin excretion, 24-hour urine, Diabetic nephropathy, Measures of albuminuria.

Introduction

Microalbuminuria (MAU) is an increased excretion of albumin in urine above physiological level. MAU has been considered the first indication of renal injury in patients with diabetes (1) and recognized as a sign of abnormal vascular function and increased vascular permeability (2). MAU is associated with a higher cardiovascular risk and higher mortality independent of other risk factors

(3). MAU is defined as a urinary albumin excretion (UAE) of 30- 300 mg/day, when measured in a 24-hour urine collection or 20- 200 μ g/min as urinary albumin excretion rate (AER) when measured in timed urine collection. It is also defined as values between 20-200 mg/L or between 30 -300 mg/g, if measured with the use of the urinary albumin: creatinine ratio (ACR) in a spot or random urine sample (1, 4, 5). Level of urine albumin below these limit are considered normal, whereas any albumin or

protein excretion above this limits represents macroalbuminuria or clinical proteinuria (1).

American diabetes association (ADA) (1) and national kidney function (NKF) (4) guidelines do not take in to account sex differences in creatinine excretion for the ACR to define MAU. For quantitative estimation of urinary albumin and defining MAU, 24-hour urine sample is considered the 'gold standard' (5, 6). However, 24-hour urine collection is inconvenient and subject to collection errors and many times it is not feasible or practically possible to collect 24-hour urine sample (e.g. O.P.D. patients).

ADA guidelines for detection of MAU permit use of 24-hour collection, timed

specimens taken over a period of less than 24 hours (e.g., overnight collection) and untimed random spot specimens (7). For random specimens, results and cut-off points for MAU must be based on either urinary albumin concentration (UAC) or the albumin: creatinine ratio (ACR) (table 1). NKF recommended the use of spot urine ACR obtained under standardized conditions (first voided, morning, midstream specimen) to detect and monitor proteinuria (level A recommendation) (8, 9). The different method for collecting urine and reporting urinary albumin are recommended by different treatment guidelines are confusing for clinicians and hamper the use of urinary albumin for managing kidney disease (10, 11).

Table 1: Cut off values used in literature for indicating normal, microalbuminuria (MAU) and macroalbuminuria (1, 4, 5).

Terms	24-hour urine Sample UAE (mg/24 hours)	Spot morning/random urine sample		
		UAC (mg/L)	ACR* (mg/g)	ACR* (mg/mmol)
Normal	< 30	< 20	< 30	< 3
Microalbuminuria	30 to 300	20 to 200	30 to 300	3 to 30
Macroalbuminuria	> 300	> 200	> 300	> 30

*ACR (mg/g) values are for both males and females (gender independent) (1, 4).

There are many studies which used spot UAC or ACR as an alternative to 24 hour UAE and demonstrated good correlation of UAC and ACR in spot urine sample with UAE in 24-hour urine sample for screening for microalbuminuria or in epidemiological studies for screening of diabetic nephropathy (11-18). It is evident from these studies (11-18) that UAC or ACR in spot / random urine sample gives accurate or similar result like 24-hour UAE. We hypothesized that UAC or ACR calculated in 24-hour urine sample will correlate with same 24-hour UAE. Primary aim of our study was to find out whether UAC and/or ACR calculated in 24-hour urine sample correlate or not with same 24-hour UAE and what is diagnostic outcome by various measures of albumin excretion calculated in 24-hour urine samples in type 2 diabetic patients?

Materials and Methods

This study was carried out in the department of Biochemistry at Krishna Institute of Medical Sciences Deemed University (KIMS DU), Karad, India, during February 2008 to March 2009. Study was approved by institutional ethical committee and informed consent was taken from all participants.

Subjects:

One hundred and twenty five known cases of type 2 diabetes mellitus (DM) attending the medicine department were taken for this study. Patients were on oral hypoglycaemic agents and regularly attending for routine follow up in diabetes. Selection of patients was non-specific, clinical history, duration and severity of disease was not taken in to consideration. Patients having past history of hospitalisation for renal dialysis and the renal transplant were excluded from study.

Urine sample collection:

All the patients were given detailed written and oral instruction about urine collection. A standard protocol was followed from sample collection. Thymol crystals used as preservative. Patients were evaluated in outpatient department (OPD) and urine collection started from next day morning. We collected 24-hour sample. No specific recommendation were made to the patients about fluid intake, diet and instructed not to do strenuous physical exercise, physical hard work prior to the urine collection.

24-hour urine sample:

Patients were instructed to collect all voided urine sample in a plastic container provided. First morning voided urine sample was discarded and time was noted. For next 24-hour urine was collected including next day's first morning void sample. Whole 24-hour urine sample of particular patient was collected in single plastic container having capacity of 5 litres. For completeness of 24-hour urine, we relied on patient's information. We have not rejected any sample due to less volume or other factors. All the samples were brought to laboratory within 6 hour of completion of collection and stored at 4°C.

Laboratory analysis:

Measurement of urinary albumin and creatinine were done on the same day of collection in clinical biochemistry laboratory, using semi-auto analyser, Erba chem-5 (Transasia bio-medicals Ltd. Mumbai, India). 24-hour urine sample was mixed properly and 5 ml of this sample was taken in to glass test tube for further laboratory analysis of albumin and creatinine. Urinary albumin was determined by immunoturbidimetry method (19 ref guy M-cross ref 35) (Tulip Diagnostics (P) Ltd. Goa, India). Values beyond the linearity limit (linearity up to

300 mg /L) were diluted with isotonic saline and retested. Urinary creatinine was measured by Jaffe's kinetic method (20) (previously 18) (Merck Specialities Private Limited, Mumbai, India). Urine albumin was quantitatively measured in undiluted urine whereas urine creatinine was determined in 1:50 diluted urine with distilled water.

Calculation and expression of data: In our laboratory urinary albumin and creatinine concentration were measured as (mg/dl), ACR was reported in (mg/g). Albumin concentration in 24-hour urine was reported as UAE (mg/24-hours). Urine albumin concentration UAC (mg/L) = Albumin (mg/dl) X 10. We calculated different parameter of albumin excretion in these patients and interpretation of each parameter was derived (table 3). Data was expressed as median and range by using Microsoft –MS office-excel.

Result

Out of 125 type 2 DM patients, 68 were males and 57 were females. The median age of the subject was 57 years. Baseline data of patients in 24-hours is shown in table 2. The median ACR was 187.30 (mg/g), UAC was 135.5 (mg/L) and UAE was 270.92(mg/24 hours) in 24-hours urine sample. Clinical outcome of nephropathy by different calculated measures (parameters) of albumin excretion in 24-hour sample is depicted in table 3. Out of 125 patients according to urine albumin concentration (UAC), 14 were having normal urine albumin, 83 were microalbuminuria and 28 were macroalbuminuria. By calculating albumin: creatinine ratio (ACR), 20 had normal urine albumin, 81 were microalbuminuria and 24 were having macroalbuminuria. By calculating 24-hour urine albumin excretion (UAE), 7 had normal urine albumin, 83 were microalbuminuria and 28 were macroalbuminuria (table 3).

Table 2: Baseline laboratory data in 24-hour urine sample (Median and Range)

	Median	Range (minimum-maximum)
AGE	57	60
Albumin (mg/dl)	13.55	186
Creatinine (mg/L)	0.76	1.24
Volume of urine (L/24hours)	2.1	2
Creatinine (g/24hours)	1.54	2.95
24-hour UAC (mg/L)	135.5	1860.00
24-hour ACR (mg/g)	187.30	2115.33
24-hour UAE (mg/24-hours)	270.92	2784.1

Table 3: Interpretation by various measures of albuminuria in 24-hour urine sample

Parameters (24-hour urine)	Normal	Microalbuminuria	Macroalbuminuria	Total
UAC	14	83	28	125
ACR	20	81	24	125
UAE	7	60	58	125

72 patient had similar interpretation either normal or microalbuminuria or macroalbuminuria by all various measures of albumin excretion in 24-hours. Out of these 7 patients had normal albumin excretion, 46 patients had microalbuminuria and 19 patients had macroalbuminuria diagnosis respectively (table 4).

53 patients showed different diagnostic interpretation by different measures of albumin excretion in the same 24-hour urine sample and divided in to five different groups (group A-E) (table 5).

Group A: Normal by both UAC and ACR but microalbuminuria by UAE.

Group B: Microalbuminuria by UAC and UAE but normal by ACR.

Group C: Microalbuminuria by UAC and ACR but macroalbuminuria by UAE.

Group D: Microalbuminuria by UAC but macroalbuminuria by ACR and UAE.

Group E: Macroalbuminuria by UAC and UAE but microalbuminuria by ACR.

Discussion

Healthy individual usually excrete small amounts of albumin (<30 mg/ 24 hours) in the urine. Increased excretion of proteins or albumin in urine is important marker for chronic kidney disease due to diabetes. MAU refers to albumin excretion that exceeds the normal range but is below the level for detection by reagent strips for urinary proteins (8). MAU is a strong predictor of diabetic nephropathy. Incipient diabetic nephropathy is suspected when

MAU is detected in urine sample of diabetic patient.

We have not used part of 24-hour urine sample as a surrogate for spot urine sample unlike Dyer et al. (6), instead we have measured albumin and creatinine in the same 24-hour urine sample and various measures of albuminuria for e.g. UAC, ACR and UAE were calculated.

For calculating 24-hour UAE we need to collect all 24-hour urine samples and volume of urine sample is needed to be known. While calculating UAC and ACR volume of urine is not required. This study was intended with the question in mind that will the UAC or ACR in the 24-hour urine correspond / correlate with the 24-hour UAE in the same sample, when urinary albumin excretion follows a circadian rhythm (5, 10) and the 24-hour urine volume is variable and many factors are affecting on this (1, 5, 21). We also know that when 24-hour urine sample available, data for albuminuria is expressed as UAE (mg/24-hour) and there is no need to calculate UAC and ACR, because 24-hour urine is considered as gold standard method to assess albuminuria /proteinuria (5, 6). In our study we found that diagnostic interpretation was different by calculating excretion in 24-hour urine sample (table 3). Out of 125 patients 72 patients showed similar interpretation either normal or microalbuminuria or macroalbuminuria and 53 patients had different diagnostic interpretation by different measures of different measure (parameter) of albumin albuminuria in the same 24-hour urine sample.

In group A, total 7 patients showed different interpretation, normal by UAC and ACR and MAU by UAE. Because total urine albumin was more than 30 mg/24-hours, hence interpretation by UAE was of MAU.

In group B, 6 patients had MAU by calculation of UAC and UAE but normal interpretation by ACR. In this group 4 (of 6) patients had UAC level of 20 mg/L, which is lower cut of value for diagnosis of MAU by UAC. Diagnosis by UAE was also of MAU. Because of the comparative low value of albumin (2.09 mg/dl) and normal value of creatinine (77.83 mg/dl), ACR was 27.33 mg/g (< 30 mg/g), hence interpretation by ACR was normal.

It is evident from our study that, even if ACR in spot urine sample having normal value, 24-hour urine sample may contain albumin more than normal range. So in such cases spot ACR may not give correct diagnostic value and 24-hour urine volume is necessary to find out correct diagnosis of various stages of nephropathy. By taking only spot urine sample the volume of 24-hour urine is not taken in to consideration. Any change / abnormality either in urine albumin or creatinine may alter the ACR. So UAC more than certain level, (we suggest UAC more than 10 mg/L) need to be confirmed with 24-hour UAE, for the correct diagnosis of nephropathy in type 2 DM.

In Group C, 24 patients showed MAU by calculating UAC, ACR and macroalbuminuria by UAE. As the albumin concentration in this group was between 20-200 mg/L, interpretation was microalbuminuria by both UAC and ACR, but total albumin excretion per day (UAE) was more than 300 mg /24 hours, which is macroalbuminuria. The reason in this group was that albumin excretion was relatively higher (>20 mg/L) and creatinine excretion was normal, urine output was also in normal range.

Group D 6 patients had MAU by UAC and macroalbuminuria by calculating ACR and UAE. By calculating UAC, interpretation was of microalbuminuria, because albumin concentration was relatively low (< 200 mg /L), creatinine concentration was lower than the normal

range. 24-hour urine volume was also in normal range. Because of the low value of creatinine ACR was high (>300 mg/g) and interpretation was of macroalbuminuria. We recommend that whenever there is low urine creatinine concentration, 24-hour urine albumin should be measured for the diagnosis of nephropathy.

Group E: 10 Patients showed MAU by calculating ACR and macroalbuminuria by both UAC and UAE. Total, albumin concentration was more, >20 mg/L, creatinine excretion was within normal range, 24-hour urine volume was also within normal range. By UAC interpretation was macroalbuminuria because the values of albumin in litre exceed the 200 mg/L (UAC > 200 mg/L is macroalbuminuria). But by ACR the ratio of albumin to creatinine was <300 mg/g (microalbuminuria) that is why diagnostic interpretation not matched with ACR. We recommend that whenever there is high albumin excretion in spot urine sample, 24-hour sample need to be collected for confirmed diagnosis as microalbuminuria or macroalbuminuria. We agree with Gansevoort RT (22) when the urinary albumin concentration exceeds a certain level (>10 mg/L) in spot or random urine sample, another 24-hour sample should be collected for measurement. When untimed random or morning urine samples are collected, results and cut off points for microalbuminuria is based on either UAC or ACR. However, both UAC and ACR also have weaknesses that may make either measure more or less preferable in a given situation (6). The major weakness of UAC is that it is affected by urinary flow rate and subjected to have greater within-person variability (5, 23).

Table 4: Average value of various parameters in 24 hours urine sample which show similar diagnostic interpretation by UAC, ACR and UAE.

Albuminuria Parameters and interpretation			Total Number Of patients	Age	Males: females	24-hour Albumin (mg/dl)	24-hour Creatinine (mg/dl)	Volume of urine (L/24hour)	24-hour Creatinine (g/24hours)	24-hour urine(UAC) (mg/L)	24-hour ACR (mg/g)	24-hour urine UAE (mg/24 hours)
UAC	ACR	UAE										
Normal	Normal	Normal	7	57.71	5;2	1.18	81.29	2.07	1.68	11.76	14.55	23.95
Microalbuminuria	Microalbuminuria	Microalbuminuria	46	59.35	28;18	9.80	72.37	2.05	1.42	97.98	162.42	185.84
macroalbuminuria	Macroalbuminuria	Macroalbuminuria	19	59.32	14;5	46.42	61.94	1.76	1.10	464.16	801.34	778.59
Total			72		47;25							

Table 5: Average value of various parameters in 24-hour urine sample which showed different diagnostic interpretation by UAC, ACR and UAE.

Groups	Albuminuria parameter and interpretation			Total number of patients	Age	Males: Females	24-hour Albumin (mg/dl)	24-hour Creatinine (mg/dl)	Volume of urine (L/24 hour)	24-hour Creatinine (g/24 hours)	24-hour urine (UAC) (mg/L)	24-hour ACR (mg/g)	24-hour urine UAE (mg/24 hours)
	UAC	ACR	UAE										
A	Normal	Normal	Microalbuminuria	7	59.28	01:06	1.51	85.57	2.29	1.96	15.14	17.87	34.10
B	Microalbuminuria	Normal	Microalbuminuria	6	58.17	02:04	2.09	77.83	2.14	1.66	20.95	27.23	44.78
C	Microalbuminuria	Microalbuminuria	Macroalbuminuria	24	56.64	11:13	16.52	77.94	2.24	1.75	165.21	230.50	369.19
D	Microalbuminuria	Macroalbuminuria	Macroalbuminuria	6	62.5	02:04	17.01	45.04	1.93	0.86	170.10	401.32	325.44
E	Macroalbuminuria	Microalbuminuria	Macroalbuminuria	10	57.9	05:05	23.79	93.70	2.05	1.93	237.90	254.65	488.78
Subtotal				53		21:32							

Weakness of ACR is that creatinine excretion varies according to gender, age, race, ethnicity (23-25) leading to problems in defining appropriate ACR cutoff values for microalbuminuria. The cost of using ACR is also higher because of the additional cost of creatinine measurements. However analytical quality, method used for creatinine estimation also affect on ACR (2, 21). However, UAC is favoured in some situations as an alternative to 24-hour albumin excretion for epidemiologic studies (6) and screening for microalbuminuria or diabetic nephropathy (18).

In summery various measures of urine albumin excretion like UAC, ACR calculated in 24-hour urine sample in type 2 diabetic patients showed different

diagnostic interpretation. We recommend when the urinary albumin concentration exceeds 10 mg/L in spot or random urine sample, another 24-hour sample should be collected for confirmed diagnosis of the stage of the albuminuria / nephropathy. We conclude that 24-hour urine sample is gold standard for diagnosis of nephropathy in diabetic patients.

Conflict of Interest None to declare

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