A Comparative Study of Clinical Utility of Spot Urine Samples with 24-h Urine Albumin Excretion for Screening of Microalbuminuria in Type 2 Diabetic Patients

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Abstract Twenty-four hour urinary albumin excretion (UAE) is considered as gold standard method for albuminuria measurement, but collection of 24-h urine is inconvenient. The aim of present study was to evaluate whether albumin: creatinine ratio (ACR) and urinary albumin concentration (UAC) in different spot urine samples correlate or not with 24-h UAE for screening of microalbuminuria in type 2 diabetic patients. We collected first morning void (FMV), random urine sample (RUS) and 24-h urine, separately on consecutive days from 104 type 2 diabetic patients. ACR and UAC in each spot urine sample compared with 24-h UAE with regard to Pearson correlation coefficient. Pearson's correlation of albumin: creatinine ratio (ACR) with 24-h UAE was ($r = 0.802$ and 0.623) in first morning void (FMV) and random

urine sample (RUS), respectively. Pearson's correlation coefficient of urinary albumin concentration (UAC) compared with 24-h UAE was ($r = 0.943$ and 0.920), in FMV and RUS, respectively, $P < 0.01$. Results revealed that values in first morning void (FMV) were better correlated with 24-h urinary albumin excretion (UAE), than the values in random urine sample (RUS). We conclude that the first morning void (FMV) may be able to replace 24-h urine collection, preferably urinary albumin concentration (UAC) in the initial screening of microalbuminuria in diabetic patients.

Keywords Diabetes mellitus · Microalbuminuria · Screening · Spot urine sample · Urinary albumin concentration

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Introduction

Patients with diabetes mellitus (DM) are at high risk of suffering renal damage. End-stage renal disease requiring dialysis or transplantation develops in approximately one third of type 1 diabetes [1]. Early detection of diabetic nephropathy relies on tests of urinary excretion of albumin. Persistent proteinuria, urinary albumin excretion (UAE) > 300 mg/day indicates frank diabetic nephropathy. Once nephropathy develops, renal function deteriorates rapidly and renal insufficiency develops. Treatment at this stage can retard the rate of progression, but not stop or reverse the renal damage [2]. A higher proportion of individuals with type 2 diabetes are found to have microalbuminuria and overt nephropathy shortly after the diagnosis of their diabetes [3].

Microalbuminuria is an early sign of incipient renal disease, a marker of its progression [4] and is one of the

most powerful predictors of cardiovascular disease in nondiabetic individuals [5]. Slightly increased excretion of albumin (15–30 mg/24 h) is both diagnostic for incipient renal disease and prognostic for the development of end-stage renal disease and mortality [6]. Microalbuminuria is now widely recognized as a sign of abnormal vascular function and increased vascular permeability [7] and associated with a higher cardiovascular risk as well as with a higher mortality independent of other risk factors [8].

Screening for microalbuminuria is currently recommended for all patients with diabetes and such screening should begin at the time of diagnosis. After the initial screening, a test for the microalbuminuria should be performed annually [3].

For quantitative estimation of urinary albumin and defining microalbuminuria, 24-h urine sample is considered the 'gold standard' [9]. However, 24-h urine collections are cumbersome and subject to error due to inaccurate timing and/or incompleteness. Even many times it is not feasible or practically possible to collect 24-h urine sample (e.g. O.P.D. patients). American Diabetes Association guidelines for detection of microalbuminuria permit use of 24-h collections, timed specimens taken over a period of less than 24 h (e.g., 4-h or overnight collection) and untimed random spot specimens [3]. For random specimens, results and cut-off points for microalbuminuria must be based on either urinary albumin concentration (UAC) or the albumin: creatinine ratio (ACR).

Few authors suggested simpler alternatives like ACR for defining microalbuminuria in spot or random collections [10–12], although some have also used UAC obtained from first morning or random spot urine samples for defining microalbuminuria [13–15]. However, it has not yet been clarified which measure for albuminuria is preferable in clinical practice [16]. There is need of simple, sensitive and reproducible laboratory procedures to screen the patients for microalbuminuria [17]. Till date there is no published study from India or using Indian subjects which compared ACR and UAC in spot urine samples with 24-h UAE for the screening of microalbuminuria in diabetes mellitus. The present study was aimed to find out whether ACR, UAC in first morning void and random urine sample correlate or not with 24-h UAE, i.e. can first morning void or random spot urine sample replace 24-h urine sample for the screening of microalbuminuria in type 2 diabetic patients.

Microalbuminuria is currently defined as a urinary albumin excretion (UAE) of 30–300 mg/24 h, if measured in a 24-h urine collection, in spot urine samples urinary albumin concentration (UAC) of 20–200 mg/l [18] and albumin: creatinine ratio (ACR) of 30–300 mg/g, in both men and women [19]. These guidelines do not take into account sex differences in creatinine excretion [3, 19], for the ACR to define microalbuminuria.

Materials and Methods

This study was carried out in the Department of Biochemistry at Krishna Institute of Medical Sciences University (KIMSU), Karad, Maharashtra, India, during February 2008 to March 2009. This project was approved by ethical committee of KIMS University.

Subjects

One hundred and four known cases of type 2 DM attending the out-patient clinic of 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 based on who was willing to provide three different spot and one 24-h urine sample.

Exclusion Criteria

Patient having past history of hospitalisation for renal dialysis and the renal transplant were excluded from study.

Urine Sample Collection

All patients were given detailed written and oral instructions about urine collection. Informed consent was taken from all patients. A standard protocol was followed for sample collection. Thymol crystals used as preservative. Patients were evaluated in out-patient clinic and urine collection started from next day morning. We collected first morning spot urine samples on two subsequent days, one random spot urine sample and one 24-h urine sample. No specific recommendations were made to the patients about fluid intake or diet, but they were instructed not to do physical exercise or physical hard work prior to the spot urine collections.

First morning void (FMV) urine sample: Patient were given plastic container and instructed to collect the first morning urine sample. After individual awakes from the sleep in the morning, first urine passed was collected. This was day 1 FMV, labelled as spot 1 (Table 1).

Table 1 Different urine specimens labelled in this study

Terms	Type of urine sample
Spot 1	First morning void (FMV) sample on day 1
Spot 2	First morning void (FMV) sample on day 2
Spot 3	Random urine sample (RUS) on day 2
24-h	24-h urine sample on day 3

Similarly on subsequent day another FMV sample was collected, this was day 2 FMV, labelled as spot 2 (Table 1).

Random urine sample (RUS): This was un-timed, non-specific random spot urine sample collected in the laboratory during day time (most of the samples received between 10.00 a.m. to 1.00 p.m). This was collected on day 2 and labelled as spot 3 (Table 1).

Twenty four urine sample: 24-h urine sample was collected on day 3. Patients were instructed to collect all voided urine sample in a container provided. First morning voided urine sample was discarded and time was noted. For next 24-h urine was collected including next day's first morning void sample (e.g. 7.00 a.m. to 7.00 a.m. next day). For completeness of 24-h urine, we relied on patient's information. All the samples were brought to laboratory within 2 h of collection.

Laboratory Analysis

Measurement of urinary albumin and creatinine were done on the same day within 6 h of collection in clinical biochemistry laboratory. Meanwhile samples were stored at 4°C. Urinary albumin was determined by turbidimetric immunoassay, by TURBILYTE-MA™ kit (Tulip Diagnostics (P) Ltd. Goa, India) on semi-auto analyser Erba Chem-Pro (Transasia Bio-Medicals Ltd. Mumbai, India). Lower detection limit of kit was 20 mg/l, and linearity up to 300 mg/l. Values beyond the linearity limit had diluted with isotonic saline and retested. Urinary creatinine was measured by Jaffe's kinetic method according to the instructions of the Merckotest® Creatinine kit [20] (Merck Specialities Private Limited, Mumbai, India), on semi-auto analyser, Erba Chem-5 (Transasia Bio-Medicals Ltd. Mumbai, India). Urine was diluted as 1:50 with distilled water before analysis. Inter-day (between days) % CV of urinary albumin and creatinine measurement was 9.3 and 2.7, respectively. The total analytical error of the ACR was 9.7%.

Statistical Analysis

Statistical analysis was performed using SPSS software package (version 15.0). Correlation between 24-h UAE (reference method) and ACR, UAC in different spot urine samples were tested according to Pearson correlation coefficient (r). Pearson correlation was considered statistically significant at $P < 0.01$ level. Baseline data was reported as median and range, age (Fig. 1) and gender distribution of patients were calculated as percentage (%). Scatter plot graphs were plotted to show correlation between two different variables (Figs. 2, 3, and 4).

Fig. 1 Age distribution of patients ($N = 104$)

Results

Table 2 shows baseline details of study subjects, calculated as Median and range.

In our study we included 104 type 2 DM patients, out of that 60 (57.7%) were males and 44 (42.3%) females. The mean age of the patients was 58.77 ± 12.16 years. The median ACR of 24-h sample was 229.34 (mg/g) and UAC was 155.15 (mg/l) suggestive of microalbuminuria, but median value of 24-h UAE was 327.88 (mg/24 h) which is suggestive of macroalbuminuria. This may be due large variation of albumin excretion in our patients. The median value of 24-h UAE may be more because of the few patients had very high amount of urine albumin excretion (UAE). This is evident from range of 24-h UAE (90.24–2805.00, mg/24 h). The median value of the ACR and UAC in all spot urine samples was suggestive of microalbuminuria (Table 2).

Figure 1 shows the age distribution of patients in our study, majority of our patients (76.9%) were above 50 years of age. In our study the spot 1 (first morning void) ACR versus 24-h UAE had the strong correlation ($r = 0.802$), $P < 0.01$ (Fig. 2a). Spot 3 (random urine sample) ACR versus 24-h UAE had poor correlation ($r = 0.623$), (Fig. 4a) as compared to spot 1. This shows that the ACR in first morning urine sample found better correlation with the 24-h UAE than the ACR in random urine sample.

When UAC in different spot urine samples compared with 24-h UAE, we found that there was a strong positive relationship between spot 1 UAC and 24-h UAE ($r = 0.943$) $P < 0.01$ (Fig. 2b). Correlation of spot 3 UAC versus 24-h UAE was ($r = 0.920$) (Fig. 4b). Our study shows that UAC in FMV was better correlated with the gold standard method (24-h UAE) than the UAC in RUS.

The Pearson correlation coefficient between ACR in various spot urine samples (correlation between same albuminuria measures in different urine samples). Spot 1

Fig. 2 Scatterplot graph of Pearson's correlation between spot 1 (FMV) and 24-h UAE. **a** spot 1 ACR (mg/g) versus 24-h UAE (mg/24 h), **b** spot 1 UAC (mg/l) versus 24-h UAE (mg/24 h)

Fig. 3 Scatterplot graph of Pearson's correlation between spot 2 (FMV) and 24-h UAE. **a** spot 2 ACR (mg/g) versus 24-h UAE (mg/24 h), **b** spot 2 UAC (mg/l) versus 24-h UAE (mg/24 h)

and spot 2 ACR had strong correlation ($r = 0.974$), $P < 0.01$ being both first morning samples on two subsequent days. This indicates that there was no much variation in daily albumin excretion in FMV sample. Spot 1 and spot 3 ACR ($r = 0.901$), spot 2 and spot 3 ACR ($r = 0.904$). All values were statistically significant ($P < 0.01$).

Correlation between spot 1 UAC and spot 2 UAC was very strong ($r = 0.992$) $P < 0.01$ being both first morning samples on two subsequent days. Correlation between the spot 1 UAC and spot 3 UAC ($r = 0.968$), spot 2 UAC and spot 3 UAC ($r = 0.961$) both values were statistically

significant $P < 0.01$. There was almost uniform creatinine excretion (g/l) in all spot and 24-h urine samples.

Discussion

There is general agreement that microalbuminuria occurs frequently in subjects with DM.

There is nearly sevenfold increase in prevalence of micro- and macroalbuminuria among diabetic subjects as

Fig. 4 Scatterplot graph of Pearson's correlation between spot 3 (RUS) and 24-h UAE. **a** spot 3 ACR (mg/g) versus 24-h UAE (mg/24 h), **b** spot 3 UAC (mg/L) versus 24-h UAE (mg/24 h)

Table 2 Patient's details ($n = 104$)

Clinical characteristics	Median	Range	
		Minimum	Maximum
24-h urine sample			
24-h urine volume (L/24 h)	2.1	0.80	2.73
Creatinine (g/24 h)	1.47	0.28	3.24
Creatinine (g/l)	0.74	0.19	1.44
ACR (mg/g)	229.34	59.17	2126.45
UAC (mg/l)	155.15	58.60	1870.00
UAE (mg/24 h)	327.88	90.24	2805.00
Spot 1 (FMV) urine sample			
ACR (mg/g)	195.79	45.36	1940.30
UAC (mg/l)	121.85	48.90	1690.00
Creatinine (g/l)	0.73	0.18	1.32
Spot 2 (FMV) urine sample			
ACR (mg/g)	198.96	46.92	1785.95
UAC (mg/l)	127.75	48.80	1652.00
Creatinine (g/l)	0.75	0.12	1.21
Spot 3 (RUS) urine sample			
ACR (mg/g)	148.7	35.89	2347.50
UAC (mg/l)	100.8	36.50	1600.00
Creatinine (g/l)	0.76	0.15	1.28

ACR albumin:creatinine ratio, UAC urine albumin concentration, UAE urine albumin excretion per day, FMV first morning void, RUS random urine sample

compared with those with normal fasting glucose [12]. Other clinical features of type 2 DM were not taken into consideration, as our aim was to measure albumin in different urine samples of same patient and to compare with 24-h UAE, irrespective of clinical condition. According to our knowledge, till date this is the first study which compared ACR and UAC from first morning void (FMV)

samples collected on two separate days and one random urine sample (RUS) with 24-h UAE.

There are many studies which compared either FMV or RUS and albuminuria measure such as UAC and/or ACR as alternative to measurement of 24-h UAE [21–27]. Few studies correlated ACR in either single FMV or RUS [17, 28, 29]. Measuring UAC alone in a spot urine sample is suggested for microalbuminuria screening by the European Diabetes Policy Group [30]. A single UAC was used to evaluate the degree of renal involvement in diabetic patients [31].

There is one recent study by Witte et al. [32], which compared FMV and spot urine sample with 24-h UAE. Our study is different from Witte et al., study as their study was conducted in a predominantly without diabetes [32].

We observed that among ACR and UAC in spot urine samples, UAC had best correlation with 24-h UAE. Our values are comparable with other studies [21, 22, 33]. According to Ahn et al., UAC had better correlation than ACR in random urine sample (RUS) when compared with 24-h albumin excretion rate [21]. Incerti et al., found UAC in RUS was best choice for the screening of microalbuminuria [22]. Our values in early morning samples are comparable to Marshal study [33]. We found correlation between UAC and ACR in random urine sample (RUS) ($r = 0.75$, $P < 0.01$) comparable with Incerti et al. [22]. The possible reasons may be that while calculating ACR, factors affecting on creatinine excretion such as age, sex, lean muscle mass, seasonal effect and biological variability were not considered in our study.

Our study is contrary to other studies:

According to Witte et al. [32], the ACR showed overall better agreement with 24-h UAE than UAC. ACR of the FMV was closely correlated to 24-h AER in insulin-dependent diabetics and was a better predictor than UAC alone [18, 34].

Strength of our study is that microalbuminuria analyses were performed in fresh urine samples without long-term storage (within 6 h of collection). We collected three different spot urine samples and one 24-h urine sample separately on consecutive days. We have taken care of recommendations of Gansevoort et al. [16].

Dyer et al. 2004 suggested UAC and ACR are acceptable alternatives to the more complex UAE, and the simpler UAC may be preferable to ACR in some respects [9]. Limitations of their study [9] was, they have used same 24-h sample as surrogate for spot morning urine sample and sample were stored frozen at -20°C for 3 years before analysis.

The explanation for why albuminuria measures in a FMV correspond better with 24-h UAE than albuminuria measures in a RUS may be that the former is less influenced by factors such as physical exercise and diet. Given the aforementioned data, we advocate collection of FMV urine samples when 24-h urine collection is not feasible.

Limitations of our study: various factors affecting on random urine sample collections such as hydration status, environmental factors (seasonal variations), and physical exercise including travelling, exact time of random urine collection were not studied. Gender difference in creatinine excretion was not considered. Data for males and females is not given separately.

As the sample size was small in our study, the conclusion need to be confirmed with large number of samples and considering all above factors. We have used ACR and UAC only for initial screening of microalbuminuria.

Urine albumin concentration and albumin to creatinine ratio are acceptable tests for population screening for albuminuria in Indo-Asians [35]. Use of albumin creatinine ratio offers no advantages and its use leads to greater heterogeneity of intra-individual variances, irrespective of urine specimen type [36]. However, if the patient produces very concentrated urine the UAC will be misleadingly high. There was almost uniform creatinine excretion (g/l) in all spot and 24-h urine sample, presumes that all the patients have a good state of hydration.

Nowadays UAC is recognized as accurate, easy to perform [37]. A single UAC measurement is easier than 24-h urine collection and eliminates the problem of incomplete collection. Moreover, it is a cheaper test for albuminuria evaluation than ACR.

Finally we conclude that UAC may be employed for microalbuminuria screening in diabetic patients. The simplicity, lower cost and better correlation of UAC with 24-h UAE justifies its preferential clinical use over ACR, and the first morning void (FMV) may be able to replace 24-h urine collection, for initial screening of microalbuminuria in diabetic patients.

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