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Original Article

Determining the Value of Albumin to Creatinine Ratio in a Single Morning Sample, Compared to the 24-Hour Urinary Albumin Excretion Test, for Determining Micro-Albuminuria in Diabetic Patients

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Abstract

Background: The aim of the study was to compare albumin to creatinine ratio (ACR) in the morning urine sample with the 24-hour urinary albumin excretion in diabetic patients by using facilities available in Iran, also determining correlation between them and day to day variation of ACR.
Methods: In this 8-month long cross-sectional study conducted in the year ending on 20 March 2006 in Zanjan, a 24-hour collected urine sample and 2 single morning samples were evaluated for measuring albumin by use of immune-turbidometry in 201 outpatients afflicted with diabetes mellitus type 2. The correlation coefficient of ACR in single samples and 24-hour urinary albumin excretion samples were assessed by use of Pearson correlation statistical test, and the regression analysis and its diagnostic function in diagnosing of micro-albuminuria.
Results: Fifty one out of 201 patients (25.40%) had micro-albuminuria and 8 (4%) were afflicted with macro-albuminuria. The correlation coefficient of ACR was maximally 81% ($P<0.0001$) and the regression equation obtained was, at the best, 24-hour urinary albumin excretion= 8.526 + (the second day ACR * 0.891). The correlation coefficient in micro-albuminuric patients was statistically significant only with respect to the second day ACR (0.50). Using the usual second day ACR cut-off of 30 mg/g, the sensitivity and specificity of 100% and 93.80%, respectively, for patients under 40 years of age (19), 47.90% and 83.30%, respectively, for 40 years, and more, of age (182) were obtained.
Conclusion: Until more assessment of other available laboratory kits is made, the morning urine albumin/creatinine ratio test will not be an acceptable alternative for measuring 24-hour urine albumin in diagnosis of micro-albuminuria in diabetic patients in Iran.

Keywords: Albumin/creatinine ratio, 24-hour urine albumin, Diabetic nephropathy, Micro-albuminuria

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Introduction

Diabetes mellitus is solely the most prevalent cause of end-stage renal diseases (1-3), such that, in the United States, diabetic nephropathy cases constitute around 40% of new cases of end-stage renal diseases (1, 2).

Micro-albuminuria is the earliest manifestation of nephropathy but, in this stage, initiation and progress of diabetic nephropathy is preventable by some interventions.

The latest edition of "Standards of medical care in diabetes" published by American Association of Diabetes(3), recommends three methods for screening micro-albuminuria: 1) measuring albumin/creatinine ratio in a random urine sample (the preferred method), in which 30 to 299 µg/gram range is considered as micro-albuminuria; 2) measuring microalbumin in a 24-hour collection of urine, which makes it possible to concurrently measure the creatinine clearance; 3) timed (e.g. 4 hours or all night) collection of urine, which is defined as micro-albuminuria in 20 to 199 µg /minute range. Even though timed and 24-hour collections of urine are standard methods of determining micro-albuminuria, they are slow and cumbersome and are amenable to errors in collecting or the problems emanating from under-emptying of bladder due to autonomic dysfunction. Therefore, the patients often have to repeat collecting (4). Regarding that creatinine corrective role for urine concentration, albumin/creatinine ratio is considered a simple indicator of 24-hour urinary albumin level (4).

Taking into account the high correlation between modulated albumin concentration, on the basis of urine creatinine in single urine samples and 24-hour albumin excretion in diabetic patients as a matter of fact, if daily creatinine excretion rate in an individual remains constant, the albumin/creatinine ratio in a single sample will reflect the daily albumin excretion (5-13).

Even though the daily excreted albumin rate is commonly estimated by use of ACR (14-16), but an important point attracts attention here: dependence of ACR on age, gender and, even, race (17-21), according to many researchers opinion, causes decrease of differentiation capacity of this ratio. Excretion of creatinine is not only lower in women compared to men but, also, diminishes with age. Therefore, ACR needs differentiating amounts specific to age and gender (22).

The objective of the present study was to compare 24-hour urinary excretion of albumin with albumin/creatinine ratio in the morning urine sample (as an accepted alternative for 24-hour urine albumin) of diabetic patients in Iran by use of the available (domestic and inside the country) facilities and determining the correlation between these two methods as well as determining the day to day variation of ACR.

Methods

This study was conducted within 8 months (from November 2005 to June 2006) and with the participation of 201 patients afflicted with diabetes mellitus type 2 in several health centres of the city of Zanjan, Iran. Three hundred four patients were selected among the diabetic patients regularly attending the health centres on the basis of inclusion and exclusion criteria of the plan (inclusion criteria: propensity to cooperation, affliction with diabetes mellitus type 1 and type 2, and exclusion criteria: women while in menstruation). After giving the necessary explanations on the objective of the study and the manner of collecting the samples and obtaining informed written consent, the questionnaires designed were filled in by the physician.

The patients attended the laboratory of Vali-e-Asr hospital of Zanjan on two consecutive days. On the first day, after

emptying the residual urine across the night at home, each of the patients delivered a single morning urine sample to the laboratory. Then, after giving necessary explanations on the manner of collecting 24-hour urine sample, the relevant containers were given to the patients and, around the same time on the second day, while receiving the second single morning urine sample, the 24-hour urine containers, too, were delivered to the laboratory.

The bacterial contamination and glucose of the urine has no effect on the measurement of albumin (22). The samples would be stable for five months at -70 ^{0C}, therefore the single samples of all patients alongside samples of 24-hour urine were kept, in separate micro-tubes, at -70 ^{0C}, until the time of performance of the concerned tests.

Use was made of the most common cut-off for differentiating normal from abnormal amounts (30 mg/g for separating micro-albuminuric individuals from the normal ones, and 300 mg/g for separating macro-albuminuric individuals from the micro-albuminuric ones) (3).

In this study, determining the urine albumin concentration was accomplished through the immunoturbidometry method and by use of the quantitative micro-albumin determination kits available inside the country, prepared by Pars Azemoun Company, with the measurement sensitivity of minimally 3 mg/litre.

The urinary creatinine concentration was measured by Jaffe method using the kits of Pars Azemoun Company.

Four normal ranges of daily creatinine excretion per kg body-weight in two groups, one above and the other below 50 years of age in terms of gender (i.e., 18.5-25 and 15.7-20.2, respectively, for men above and below 50 years of age, and, in the same way, 16.5-22.4 and 11.8-16.1 for women) (23) were used for examining the daily creatinine excretion position of each individual in the normal range in terms of age, weight and gender, in order to determining the sufficiency of 24-hour urine sample collection.

The biochemical measurements mentioned above were performed by use of Selectra 2 auto-analyser manufactured by Vitallab of Holland. The qualitative measurement of glucose and protein were accomplished using Combi-Screen urine tape manufactured by Analyticon of Germany. The assessment by use of urine tape was made only on the first urine sample of the individual.

The study data were analyzed by use of SPSS Statistical Software version 11.5 and through regression and Pearsson correlation coefficient statistical tests. $P \leq 0.05$ was considered statistically significant.

In the last stage, the receiver operating characteristic (ROC) curves for assessing the general ability of the above-said tests in determining micro-albuminuria were drawn. The surface under the 1:00 curve was considered as ideal and the surface under the 0.50 curve was considered as lacking any value.

Results

Considering the competence of 24-hour urine sample collections, the urine samples of 201 patients were examined. Using 30-299.99 mg daily urinary albumin excretion for definition of micro-albuminuria, 51 out of 201 patients mentioned above (25.4%) were afflicted with micro-albuminuria and 8 (4%) were afflicted with macro-albuminuria (albumin excretion $\geq$ 300 mg/day). Most micro-albuminuric individuals (43 patients, 84.30%) had no pyuria (WBC$\geq$10/HPF).

The demographic data related to each of these three patient groups have been summarized in Table 1.

The correlation coefficient for each of the first (ACR1) and second (ACR2) morning samples and the mean of the two (ACRm) were 0.24 ($P<0.001$), 0.81 ($P<0.0001$) and 0.56 ($P<0.0001$) , respectively. The regression equation was obtained as

follows: daily excreted albumin in 24-hour collected urine = 0.891 ACR2 + 8.526.

The correlation coefficient in micro-albuminuric patients (51 numbers) was, also, calculated for each of the above-said ratios, respectively, as 0.16, 0.50 and 0.27, which was statistically significant only with respect to ACR2 (P<0.0001). By segregating the first samples containing 10 or more white blood cells in the high power field microscopy (pyuria), which comprises 18 samples out of 201 patients, the correlation coefficient related to AER and ACR1 increased from 0.24 in non-pyuric samples (P=0.001) to 0.55 (P=0.018) in pyuric ones.

The following results were obtained for ACR in the first and second samples and the mean of the two.

1. The ACR function in the first morning sample in determining micro-albuminuria: 39.20% sensitivity, 85.20% specificity, positive predictive value =46.5 %, negative predictive value = 81.20%, accuracy = 73%
2. ACR function in the second morning sample in determining micro-albuminuria: 51% sensitivity, 84.50% specificity, positive predictive value = 52%, negative predictive value = 83.30%, accuracy = 75.60
3. The mean ACR function in the first and second days samples in determining micro-albuminuria: 47.10% sensitivity, 81.70% specificity, positive predictive value = 47%, negative predictive value = 82.30%, accuracy = 72.50%

In case of making use of different cut-offs for the urinary albumin/creatinine ratio for differentiating normal from micro-albuminuric individuals, one can obtain different sensitivities and specificities for this test the result of which is ROC curve (Figure 1). By use of ROC curve in the patients under study, excluding the macro-albuminic individuals (Figure 1), at the best, by use of a 25 mg/g cut-off the sensitivity and specificity obtained, were, respectively, 62.70% and 79.60%.

By use of under the 0.725 curve at the best cut-off 12.2 mg/g were obtained the sensitivity and specificity, 73% and 84%, respectively for men. For women we used under the 0.805 curve at the best cutoff 29.4 mg/g and were obtained the sensitivity and specificity, 72% and 70%, respectively.

In case of making use of the usual cut-off of 30mg/g for the second ACR in all 201 patients, the following values were obtained: 100%, 93.80%, 75%, 100% and 94.70% for patients < 40 years of age (n = 19) and 47.90%, 83.30%, 50%, 81.40% and 73.50% for patients ≥ 40 years of age (n = 182), respectively, for sensitivity, specificity, positive predictive value, negative predictive value and accuracy.

Calculating [ACR1 – ACR2] x 100/ACR1 separately for all patients and, then, determining the mean of these values in all patients under study (n = 201), a mean of day to day variations of 554.60% (i.e., a mean 5.5 times the increase or decrease in two morning samples) with a SD of 5014.90 was obtained.

The correlation coefficient of ACR1 and ACR2 was 0.177 (P = 0.012).

Table 1.The demographic data of patients having sufficient 24-hour urine sample (201 individuals) as separated according to the relevant groups from the viewpoint of daily albumin excretion (mean± standard deviation)

	Division on the Basis of Daily Urinary Albumin Excretion			
Variable	**Normal**	**Micro-albuminuric**	**Macro-albuminuric**	**Total**
Age (year)	53±11	54±11	59±9	53±11
Genders Number (male/female)	44/98	18/33	4/4	66/135
History of Diabetes (Year)	4.6±4.3	5.4±4.4	9.6±6.7	5±4.5
BMI (Kg/m^2)	27.6±4.2	27.8±4.2	25.3±3.4	27.6±4.2

Data are means ± SD

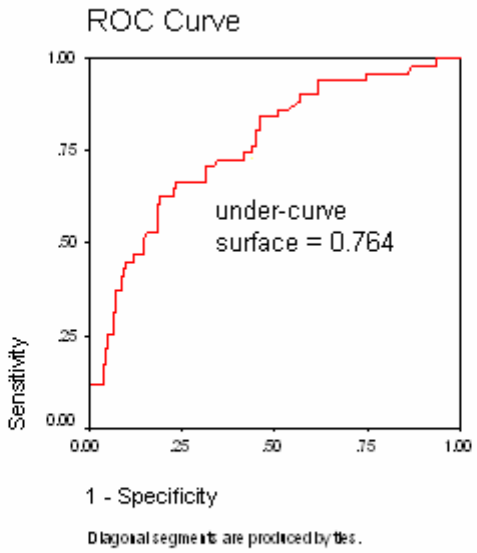

Figure 1. ROC curve for assessing different cut-off functions for the albumin to creatinine ratio related to the second urine sample in determining micro-albuminuria.

Discussion

Daily albumin excretion rate has not acceptable correlation with ACR in single urine sample while the correlation with daily albumin excretion in the collected 24-hour urine in micro-albuminuric patients decreases, which appears to be due to considerable decrease of the number of samples compared to the assessment of correlation in all patients. Such difference was mentioned in at least two similar studies (11, 24). In one of these studies (11) it was noted that these correlations decreased more in normo-albuminuric individuals which, some consider the low albumin to total protein ratio in the lower protein amount in urine as its cause.

The ACR correlation coefficient obtained with the daily albumin excretion in 24-hour urine, which was, at best, in the second day morning sample (0.81), considerably differs from some coefficients mentioned in the references. These coefficients in all patients (either micro-albuminurics or others) vary from 0.88 to 0.98 (4, 8, 14, 24-26). Irrespective of the fact that in some cases use is made of Spearman correlation coefficient instead of the Pearson one, the communities under study were much similar to our target community and majority of them use was made of immuno-turbidometry method for measuring albumin. Also, most samples were similar to our sample from the viewpoint of number. In one case, the correlation coefficient in albuminuric patients has been mentioned separately (R = 0.55) (27). It is interesting that, here, the correlation of albumin concentration in random urine sample (before and after dividing it by the creatinine concentration) with daily albumin excretion in 24-hour urine has virtually not changed (from 0.80 to 0.81). Of course, this is not a unique point; it is a reason why some investigators make use of albumin concentration, without any need to divide creatinine, for estimating the daily albumin excretion in 24-hour urine collected and determining micro-albuminuria (13).

The first day morning sample had a weaker correlation with the second day one. For finding the cause, the mean of each of the ratios in all patients was taken into account. Examining the means of albumin and creatinine concentrations showed that they were all decreased in the second sample (52% and 9% respectively) but, as the decrease of mean of albumin concentration is clearly more than the decrease of creatinine concentration, one can justify the considerable ratios under study in the second sample. Because the tests on both samples of an individual were performed at the same time and under the same circumstance, the reason should be sought in the features of the samples, which may be due to the more possibility of emptying the residual urine in the bladder before preparing the second day morning sample (this is because the patient had been collecting the 24-hour urine before attending the laboratory). Thus, the second sample should be more dilute.

These points mentioned can justify the considerable difference between the means of the first and second samples in the total individuals participating in the study, in addition to shortage of morning samples.

The cut-offs mentioned in scientific references for segregating micro-albuminuric patients are numerous and each research group introduces figures on the basis of the sample under study and ROC emanating from it (4, 14, 15, and 28). Similar to this rule, in our study by use of the widely-practiced cut-off of 30 mg/g were obtained the sensitivity and specificity of 51% and 84%, respectively, and by use of the separate cutoffs of 17 and 25 mg/g that recommended for males and females respectively, were obtained the sensitivity and the specificity of 69% and 77% (27).We should be pay attention to this point that these figures are not acceptable for screening test (the acceptable figures for screening test in both sexes should be more than 80%) (4). The under-curve surface of ROC (totally 76%, and 80% for men , 72% for women), too, demonstrates that, in the most desired state, the sensitivity and specificity above 75% (both being the same) with positive predictive value, negative predictive value and accuracy of 49%, 88% and 74%, respectively, will not be discerned by use of separate cut-offs of 12.20 mg/g for men and 29.40 mg/g for women (the best general cut-off is 25 mg/g, with a sensitivity of 63% and a specificity of 80%).

Chaiken and colleagues (24), obtained sensitivity, specificity, positive predictive value, negative predictive value and accuracy of 92%, 97%, 88%, 98% and 96%, respectively, for ACR in determining the daily albumin excretion in 24-hour urine sample, by use of the widely-practiced cut-off of 30-300 mg/g in 123 samples of patients afflicted with diabetes mellitus type 2, using micro-albuminuric samples and laboratory techniques similar to those used in the present study.

By segregating the individuals to those less than 40 years of age and those at or more than 40, the test function improves considerably (the sensitivity of 100% and the specificity of 87-94%), but, due to low number of individuals less than 40 (19 individuals), this result is not reliable.

In examining the day to day variations, many differences are observed among the values of two morning samples which appear to emanate from two factors:

1. The important point is that the differences are measured in two consecutive samples, while, in the available tests related to variability and reproducibility of some tests under study (14, 29 and 30), at least 5 samples from each individual have been assessed and, on this basis, the correlation coefficient or the variations coefficient have been determined.
2. Considerable variations are observed in albumin concentration (in the first day, 10.80 ± 46 and, in the second day, 5.20 ± 11.60) and creatinine concentration (in the first day, 147.30 ± 150 and, in

the second day, 133.50 ± 78.20) between two morning samples. As is seen, all variables in the second sample, compared to the first one, diminish, which are more obvious in albumin concentration (a decrease of 52%). The above-mentioned variations lead to difference between the albumin to creatinine ratios in two samples (in the first day, 90.10 ± 322.40 and, in the second day, 50.90 ± 125.70).

The limitations of the present study are as follow: lack of assessment of at least 5 single samples in order to better examine the day-to-day changes of micro-albumin, the settlement of which was impossible, taking into account the difficulties of the patients' attending the laboratory and the inadequate budget of the plan.

In any way, the extreme weakness of the ACR, as a distinct and first rank alternative for the daily excreted albumin in the 24-hour collected urine, to be used in determining micro-albuminuria in the present study, is to be considered hesitatingly.

Taking into account the main weakness observed in this study concerning the diagnostic value of ACR, having been expected to be the best, in determining micro-albuminuria, it appears that the questionable points in this regard are the creatinine measurement method particularly with respect to the chemical reagents (kits) used domestically and, also, the measured concentration of albumin. Of special note is that the domestically produced kit has been recently introduced to Iranian market.

On the basis of this study, it appears that, in the current condition of Iran, ACR cannot be a convenient alternative for measuring the 24-hour urine albumin excretion in diagnosing the micro-albuminuric diabetic patients. Therefore, it is proposed that, until the comparison of the function of the kit present for determining urine creatinine is performed, use be made only of the measurement of 24-hour urine albumin for this purpose. Regarding the importance of the subject, the assessment of the domestic laboratory kits being used with the aid of the universally creditable kits, with the existing more accurate methods and, also, by increasing the number of the single samples (minimally 5 samples) for creating the circumstances for determining the repetitiveness of the tests, their variation coefficients and, also, examination of more extended sample size in the future studies are recommended.

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