

ROLE OF URINARY ALBUMIN TO CREATININE RATIO AND SPOT ALBUMINURIA IN PREDICTING SIGNIFICANT ALBUMINURIA IN PATIENTS OF DIABETIC NEPHROPATHY

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ABSTRACT: INTRODUCTION:Diabetic nephropathy is the most common cause of End Stage Renal Disease (ESRD). The earliest clinical evidence of nephropathy is microalbuminuria. The commonly used investigations to diagnose microalbuminuria include 24 hours urinary Albumin Excretion Rate (AER), Albumin to Creatinine ratio (ACR) and spot microalbuminuria. These latter two methods have been found to be convenient, cost effective and efficient in screening patients of microalbuminuria when compared with 24 hour collections.**AIM OF STUDY:** The present study was conducted to know whether ACR or spot microalbuminuria alone can predict and quantify microalbuminuria in patients with Diabetic Nephropathy.**METHODOLOGY:**100 known patients of type II DM were enrolled for the study. 24 hour urinary sample was taken to quantify albuminuria and spot urine sample was taken for ACR and microalbuminuria. The results obtained by ACR and spot microalbuminuria were compared with the gold standard 24 hours urinary protein measurement.**RESULTS:**Sensitivity and specificity for predicting true microalbuminuria were found to be 91% and 75% respectively for ACR. Its positive predictive value was 57% and negative predictive value was 90%. Sensitivity and specificity were found to be 92% and 82% for spot microalbuminuria with positive predictive value of 40% and negative predictive value of 95%.**CONCLUSION:**The low specificity and low positive predictive value of ACR and spot microalbumin does not support their use for quantifying urinary albumin excretion and it was concluded that these methods alone cannot be relied upon for quantification of albuminuria. However the high sensitivity and negative predictive value of the tests suggests that these are good routine outdoor screening tests for finding out those patients who should undergo quantification by 24 hours urine albumin excretion.

KEYWORDS:Albumin to Creatinine Ratio, Spot microalbuminuria, 24-hr urinary protein, Diabetes Mellitus, End Stage Renal Disease

INTRODUCTION: Diabetic nephropathy is currently the most common single cause of End Stage Renal Disease (ESRD).¹ This may be because i) Prevalence of Diabetes, particularly type II is increasing. ii) Longer life span of Diabetics. The earliest clinical evidence of nephropathy is the appearance of low but abnormal levels (>30 mg/day or 20µg/min) of albumin in urine, referred to as microalbuminuria and patients with microalbuminuria are diagnosed to have incipient nephropathy. Without specific intervention, approximately 80% of subjects with Diabetes who have sustained microalbuminuria have their urinary albumin excretion rise at a rate of 10-20% per year reaching the stage of overt nephropathy or clinical albuminuria (>300 mg/24 hrs. or >200 µg/min) over a period of 10-15 years.^{2,3,4}

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Routine urine analysis should be done yearly in Diabetics. If positive for proteins, a quantitative measure is frequently helpful in development of treatment plan. If urine analysis is negative for proteins, a test for the presence of microalbumin is necessary. Screening for the same can be performed by three methods.

- 1) Timed, e.g. 24-hr Albumin Excretion Rate (AER)
- 2) Measurement of Albumin to Creatinine Ratio (ACR) in a random spot urine collection.
- 3) Spot microalbuminuria

The need of a 24 hour collection is as a result of the high degree of variation in the urinary protein excretion during the course of the day. This precludes the use of shorter collection time or the use of random urine sample for protein measurements. Depending on diurnal variation, the value may vary from 100-500%. This diurnal variation may be due to i) variation in water intake and excretion, b) rate of diuresis iii) exercise iv) recumbence and e) diet.⁵ However because of inconvenience of 24 hr. urine collection, the ACR and spot microalbuminuria have been adopted to estimate urine albumin excretion rate. The ACR has been found to be convenient, cost effective and efficient in screening patients of microalbuminuria when compared with 24 hr. collections. Now a days ACR is used extensively to determine the presence of microalbuminuria and has even been used as a gold standard when assessing the efficacy of other diagnostic tests.^{6,7,8} However some say that protein/creatinine ratio which is a quantitative test should be used additionally in monitoring progression of renal disease.⁹

The present study was undertaken to know whether ACR or spot microalbuminuria alone can predict and quantify microalbuminuria in patients with Diabetic Nephropathy.

AIM OF THE STUDY: The present study was conducted to know whether urinary Albumin to Creatinine Ratio or spot microalbuminuria can reliably predict and quantify significant albuminuria in patients of Diabetic Nephropathy.

MATERIALS AND METHODS: The study was done on 100 patients diagnosed with type II Diabetes Mellitus visiting medicine department of a tertiary care institute. The study was approved by college ethical committee. Informed written consent was obtained from all the participants. Their urine sample was taken for measurement of 24 hour urinary albumin, first morning sample was discarded and patient was asked to collect complete 24 hour sample including next morning sample in a clean sterile container. For estimation of albumin to creatinine ratio and spot microalbumin, random morning sample was taken in a clean sterile container.

24 hour urine albumin was measured by immunoturbidimetric method. ACR was measured by Clinitek urine analyzer (Siemens). It measures albumin and creatinine in the spot urine sample and calculates the ACR. The test is based on formation of a strong blue coloured complex between albumin and the high affinity dye bis (3, 3 diiodo 4, 4 dihydroxy 5, 5 dinitrophenyl 3, 4, 5, 6 tetrabromosulfonephthalein). At constant pH the development of blue color is due to albumin. Creatinine measurement is based on peroxidase like activity of a copper containing complex that catalyzes the reaction of diisopropyl benzene dihydroperoxide and 3, 3, 5, 5 tetramethyl benzene. The color form varies from green to blue.¹⁰

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Spot microalbumin was measured by Nycocard reader which is based on the principle of solid phase sandwich format immunometric assay.¹¹ Both clinitek urine analyzer and nycocard reader are point of care testing instruments.

RESULTS: Routine investigations were done in all patients to rule out any overt renal abnormality. Serum creatinine, blood urea and serum electrolytes were measured in all the patients.

The criteria of microalbuminuria and macroalbuminuria followed was as laid down by national evidence based guidelines for diagnosis, prevention and management of chronic kidney disease in type 2 diabetes and is given below in Table no. 1.

| | 24 hour Albumin excretion rate(AER) | Albumin to Creatinine Ratio(ACR) | Spot Urinary microalbumin |
|------------------|-------------------------------------|--|---------------------------|
| Microalbuminuria | 30-300 mg/24 hour | 2.5-25mg/mmol in males 3.5-35mg/mmol in females | 30-300 mg/l |
| Macroalbuminuria | >300 mg/24 hours | >25 mg/mmol in males >35 mg/mmol in females | >300 mg/l |

Table no. 1: Showing criteria of microalbuminuria and macroalbuminuria

The sensitivity, specificity, positive and negative predictive values of ACR and spot microalbumin as compared to 24 hour urinary albumin excretion are given in table no:- 2 and figures 1 & 2.

| Parameter | Sensitivity | Specificity | Positive predictive value | Negative Predictive value |
|-------------------|-------------|-------------|---------------------------|---------------------------|
| ACR | 91% | 75% | 57% | 90% |
| Spot microalbumin | 92% | 82% | 40% | 95% |

Table 2: Showing, sensitivity and specificity positive predictive value, negative predictive value of ACR and spot microalbumin

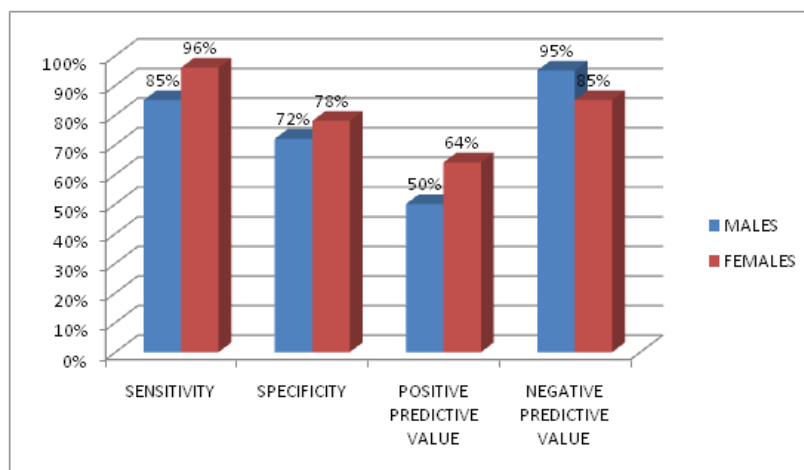


Fig. 1 showing sensitivity, specificity, positive predictive value and negative predictive value of Albumin to Creatinine Ratio in males and females.

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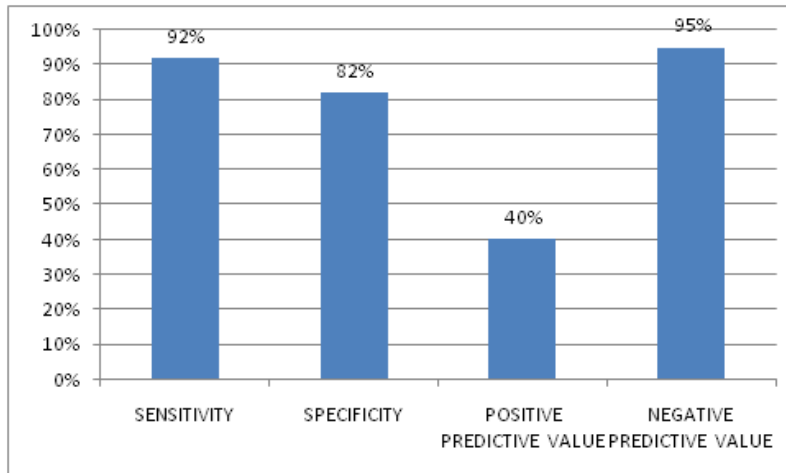


Fig. 2 showing sensitivity, specificity, positive and negative predictive values of spot microalbuminuria

Sensitivity and specificity of ACR and spot microalbuminuria at different cut off value are given in tables 3 and 4.

| Cut off level(mg/mmol) | Males | | Females | |
|------------------------|-----------------|-----------------|-----------------|-----------------|
| | Sensitivity (%) | Specificity (%) | Sensitivity (%) | Specificity (%) |
| 8.0 | 55.2 | 98.4 | 77.9 | 99.3 |
| 6.0 | 63.2 | 96.0 | 77.5 | 95.7 |
| 4.0 | 76.3 | 91.8 | 86.6 | 90.8 |
| 2.0 | 87.5 | 88.9 | 93.0 | 80.1 |
| 1.0 | 97.0 | 63.1 | 92.8 | 50.4 |

Table 3: showing sensitivity and specificity of Albumin to Creatinine ratio at various cut off values

| Cut off level (mg/l) | Sensitivity (%) | Specificity (%) |
|----------------------|-----------------|-----------------|
| 200 | 30 | 100 |
| 100 | 50 | 98 |
| 70 | 73.1 | 95 |
| 40 | 91.9 | 89 |
| 20 | 92 | 82 |
| 10 | 97 | 54 |

Table 4: showing sensitivity and specificity of spot microalbuminuria at various cut off values

DISCUSSION: Proteinuria is recognized as an independent and fundamental risk factor for cardiovascular and renal disease and as a predictor of end stage renal disease in Diabetics. Detection of an increase in protein excretion is known to have both diagnostic and prognostic value in the initial detection and confirmation of renal disease. Microalbuminuria is not only the earliest manifestation of nephropathy; albuminuria is a marker of greatly increased morbidity and mortality with either type I or type II DM.¹²

The commonly performed tests for albuminuria are timed urinary collection like 24 hours urinary protein, and spot urinary collections for microalbumin and Albumin to Creatinine Ratio. Besides this urinary dipstick is commonly done test to rule out proteinuria. However it has minimal to nil value in the quantification of urinary proteins, because it depends not only on the amount of protein but also on the volume of urine at the time of testing.¹³

24 hours urine collection, which is the gold standard used to estimate proteinuria has its own drawbacks like it is cumbersome and there is problem in accuracy of collection which depends on patient.^{9,14} In order to overcome the 24 hrs. collection procedures, the scientists ultimately discovered methods like single voided spot urine Albumin: Creatinine ratio in the presence of stable Glomerular Filtration Rate. The use of single voided urine ACR as an alternative to 24 hr. urine collection was suggested first in 1980.¹⁵

In the present study sensitivity and specificity of ACR was found to be 91% and 75%, with the positive predictive value of 57% and negative predictive value of 90%. The low specificity and positive predictive value indicate more chances of false positive test and precludes the use of ACR as the only test required for quantification of microalbuminuria.

Similar low specificity values for ACR as a predictor of microalbuminuria were found in other studies also. Derhaschnig et al found sensitivity of 85.7%, specificity of 91%, Positive Predictive Value as 42.9 and Negative Predictive Value as 98%.¹⁶ In a study by Houlihan et al the sensitivity was found to be 95.7% in males and 93.35% in females.¹⁷

The sensitivity at different cut off levels of ACR in the present study was found to vary from 97% at cut off of 1mg/mmol to 55% at 8mg/mmol in males and in females it was found to be 92% at cut off of 1 mg/mmol and 78% at cut off of 8mg/mmol. The specificity was 63% at 1 mg/mmol and 98% at 8mg/mmol in males and 50% at 1mg/mmol and 99% at 8mg/mmol in females indicating its role in diagnosing normoalbuminuric patients at lower range and patients with high microalbuminuria at higher range.

The ACR has been found to be convenient, cost effective and efficient in screening patients with albuminuria because of high negative predictive value however because of low positive predictive value, the utility of ACR in predicting true microalbumin excretion is low. It is thus suggested that ACR is a good method for selecting patients who should collect timed urine samples for albumin excretion measurement.

These findings demonstrate that the performance of ACR as an estimate of microalbumin excretion was suboptimal when compared with 24-hr AER. A major implication of this result is that a decision to prescribe lifelong medication in an effort to prevent nephropathy may be wrong one third of times if it is based on ACR alone.¹⁸

The ACR did have a high negative predictive value, indicating that the ACR is useful in determining when the patients are normoalbuminuric again similar to the spot microalbuminuria, but still it cannot be relied on when making a therapeutic decision such as diagnosing early stage

diabetic nephropathy. It was suggested that the inability of the ACR to predict microalbuminuria may be due to i) variations in urine volume, which affects the concentration of creatinine and/or albumin within a sample. As a result, inconsistent urine collection in random sample can result in increased variability in ACR results ii) Presence of unstable GFR as creatinine excretion rate depends on a stable GFR.¹² The protein excretion would also likewise be fairly stable. Hence the ratio of the two in a single voided sample would reflect the cumulative protein excretion over the day as the two stable values would cancel out the time factor.¹⁵ The correlation between 24 hr. urinary protein and ACR was not found to be convincing in the patients who have low GFR (GFR \leq 15).¹⁹

Age also plays an important role in this as creatinine excretion decreases with age. Thus age adjusted ACR could result in higher specificity. It has been studied that spot ACR false positivity increased from the 15.9% (age 40-65 yrs.) to 31.8% (age>65yrs) in men and from 10.5% (age 40-65 yrs.) to 28.3% (age>65yrs) in women. Houlihan et al found spot ACR to be a good screening test for microalbuminuria, but a poor predictor of quantitative AER, and suggested that it should not be used as a diagnostic test.¹⁷

In the present study spot microalbuminuria was having sensitivity of 92% and specificity of 82% in diagnosing true microalbuminuria. The positive predictive value was 40% and Negative Predictive Value was 95%. The sensitivity was maximum at lower cut off values of microalbumin while specificity was least and it was 100% at cut off 200 mg/l. At the suggested cut off value of 20mg/L the spot urinary albumin has been found to be quite sensitive with a high negative predictive value, clearly indicating its importance in diagnosing normoalbuminuric patients. Similar findings were given by Derharnig et al. with sensitivity of 91.5%, specificity of 84.3%, positive predictive value of 44.2 % and negative predictive value of 97.9%¹⁶

To summarize, the limitation of both ACR and spot microalbuminuria are their low specificity and positive predictive values and hence chances of false positive tests are quite high which may be contributed by various factors like age, circadian rhythm of proteinuria and unstable GFR.

CONCLUSION: ACR and spot microalbuminuria have sensitivity of 91% & 92% and specificity of 75% & 82% respectively with low positive predictive value and high negative predictive values. Thus we conclude that these tests can reliably predict significant albuminuria in patients of Diabetic nephropathy, but none of these can replace the need for quantification by 24-hour urine albumin excretion. However because of convenience of sample collection and relatively high sensitivity and high negative predictive value, and as both the instruments are point of care testing instruments these tests can be used as screening tests to find out which patients should undergo 24 hour albumin excretion test.

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