

Efficacy of Spot Urinary Albumin Excretion Test for the Detection of Early Nephropathy

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ABSTRACT

Objective: To compare the effectiveness of spot urinary microalbumin excretion for detection of early diabetic nephropathy in comparison with 24 hours urinary proteins in patients with diabetes mellitus.

Materials and Methods: The study was conducted at Nephrology Department of Pakistan Institute of medical Sciences, Islamabad. It included diagnosed cases of type 1 or 2 diabetes mellitus, (with type 2 diabetes of any duration and type 1 diabetes of more than 5 years duration) who tested negative for overt albuminuria on standard urinary dipstick done on two occasions. Each patient was provided a plastic container with capacity of 4000 ml for 24 hours collection of urine. The study group subjects were requested to provide early morning urine sample for spot urine test. Urinary microalbumin was determined by the immunoturbidimetric method. 24-hour urinary albumin excretion was determined by photometric test according to biuret method. SPSS version 12 was used to record and analyze the gathered data. Descriptive statistic and frequencies of the spot urine albumin and 24 hours urinary albumin were measured. The sensitivity, specificity, negative predictive value, positive predictive value, accuracy and efficacy of spot urine microalbumin test was calculated in comparison to 24-hour urine protein test.

Results: Out of 289 patients of Diabetes mellitus included in the study, 39 (13.5%) had type 1 Diabetes mellitus and 250 (86.5%) had type 2 Diabetes mellitus. The mean age of the patients was 54.07±9.5 years. Among total patients 113 (39.1%) patients had positive 24-hour urine protein and 98 (33.9%) had positive spot urine microalbumin test. The sensitivity, specificity, positive predictive value and negative predictive value of the spot urinary microalbumin test in comparison to 24-hour urine protein test was 83%, 97.7%, 96% and 90% respectively. In total spot urinary microalbumin test was accurate in 92% cases. When the results of spot urine microalbumin test and 24-hour urinary protein test were compared using the chi-square test, it was found that patients with a positive spot urine microalbumin test had a statistically significant probability of having a confirmed diabetic nephropathy on 24-hour urinary protein test; p= 0.00.

Conclusion: Spot urine microalbumin test is a highly sensitive and specific tool in the diagnosis of early diabetic nephropathy with high positive predictive value. Spot urine microalbumin test offers the advantage of speed, simplicity and early diagnosis. Spot urine microalbumin test has acceptable accuracy as compared to 24-hour urine protein test.

Key words: Albuminuria, Diabetes mellitus, Microalbuminuria, Nephropathy, Proteinuria, Spot urinary albumin

Author's Contribution

¹Active participation in active methodology, Interpretation and discussion ²Synthesis and Planning of the research, Conception, Review the Study, ^{3,4} Review and paper writing

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Introduction

Diabetes Mellitus (DM) is one of the major illness affecting 284 millions of people worldwide. It caused approximately 88014 deaths in Pakistani population at the end of year 2010, according to International Diabetes Federation.¹ Diabetic nephropathy affects 25 percent of patients with diabetes that progress to chronic renal failure.² It occurs both in type 1 and type 2 DM. Diabetic kidney disease presents in its earliest stage with microalbuminuria (defined as albumin-to-creatinine ratio between 30-299 µg albumin/milligram of creatinine) in the urine, also called as incipient nephropathy. With the disease progression, urine albumin levels increase until the patient develops overt proteinuria (defined as more than 300mg per 24 hours or more than 200 mcg per minute). Overt proteinuria is followed by a gradual decrease in glomerular filtration rate that ultimately leads to kidney failure.³

Increased urinary protein excretion is the earliest clinical manifestation of diabetic nephropathy.^{4,5} However, when assessing protein excretion, the urine dipstick is a relatively insensitive marker for initial increases in protein excretion, not becoming positive until protein excretion exceeds 300 to 500 mg/. Using a specific assay for albumin is a more sensitive technique. The normal rate of albumin excretion is less than 20 mg/day (15 µg/min); persistent albumin excretion between 30 and 300 mg/day (20 to 200 µg/min) is called microalbuminuria and, in patients with diabetes (particularly type 1 diabetes), is often indicative of early diabetic nephropathy, unless there is some coexistent renal disease. Protein excretion above 300 mg/day (200 µg/min) is considered to represent macroalbuminuria (also called overt proteinuria, clinical renal disease, or dipstick positive proteinuria).⁶ In general, immunoassay methods are the standard for measurement of microalbuminuria, and comprise four different techniques. These include, RIA (radioimmunoassay), ELISA (enzyme-linked immunosorbent assay), RID (radioimmunodiffusion) and Immunoturbidometry. These four methods have a similar level of sensitivity and specificity, and are used in different areas according to the resources available.⁷ Immunoturbidometry is more frequently used because of its greater simplicity.

Microalbuminuria is considered as a reliable predictor of nephropathy in diabetic patients. Early detection of microalbuminuria is pivotal because the condition at this stage is potentially reversible, and appropriate treatment may prevent progressive diabetic renal damage.⁸ Diagnosis of microalbuminuria can be carried out by number of approaches however three most common methods used are measurement of albumin-to-creatinine ratio on a spot urine test, albumin from a 24-hour urine collection, and albumin from a timed collection (e.g.10 hours overnight).⁹ Twenty-four hours' quantitative assessment of albuminuria is considered as gold standard but it is time consuming and sometimes difficult for the patient to understand the voiding and collection precautions.¹⁰ Spot urinary test is practical with negligible collection errors. Role of spot urine test in the primary prevention of diabetic nephropathy is clearly understood to detect the change from normal urinary albumin excretion to microalbuminuria with regular follow up.¹¹

Predictive power of urinary proteins, changes over the time as the disease progresses. Positive test indicates disease duration of more than 05 years.¹² There is no definitive "cure" for diabetic nephropathy, but if it is diagnosed at early stage, its progression can be slowed down with good glycemic and weight control, optimized blood pressure and other factors effecting its progression.¹³ This study was designed to evaluate the efficacy of spot urinary albumin excretion for detection of early diabetic nephropathy in comparison with 24 hours urinary proteins in patients with diabetes.

Operational Definitions

Diabetic nephropathy: It is a condition in diabetic patients that damages on kidney function. It starts with microalbuminuria, proteinuria and progresses to kidney failure. If we can detect microalbuminuria early then we can slow down the disease process, even reverse it in microalbuminuria stage. This is the stage in diabetic nephropathy when overt proteinuria is not present i.e. the routine dipstick proteinuria will be absent. However, they may have microalbuminuria detected on either 24-hour urine sample or spot urine microalbuminuria testing. The urine dipstick is also a relatively insensitive marker for

initial increases in protein excretion, not generally becoming positive until protein excretion exceeds 300 to 500 mg/day.

Spot urinary albuminuria

It is an immunoturbidimetric technique by which microalbuminuria can be detected in a spot urine sample.

24 hours urinary albumin

It is the gold standard method to calculate the renal loss of protein over 24 hours for the correct estimation of the proteins to assess the renal failure.

Patients and Methods

This cross-sectional study was conducted at Nephrology Department, Pakistan Institute of medical Sciences, Islamabad, over 6 months' time. In total 289 patients diagnosed with type 1 Diabetes (of more than 5 years duration) or type 2 diabetes of any duration, of either gender and negative for overt albuminuria on standard urinary dipstick done on two occasions were included in the study. Patients with overt proteinuria, known chronic kidney disease, patients with urinary tract infections, hematuria of any cause, nephrolithiasis, hypertension, febrile illness, patients recently using nephrotoxic drugs, chronic liver failure, hepato-renal syndrome, organ transplant, kidney transplant, liver transplant, immunocompromised states, HIV-AIDS, patients with known malignancy, those on chemotherapeutic agents and pregnant females were excluded from the study

Data were collected on a specially designed performa. Institutional ethical committee approved this study and a written informed consent was obtained from all subjects.

A random sample of 289 subjects with type 1 & 2 DM were included in the study, provided they were negative on routine urinary dipstick testing on two occasions in their initial outdoor visit using (Multistix 10, SG, Bayer, Bridgend, UK. Each Demographic details were documented. Each patient was provided, a plastic container with capacity of 4000ml for 24 hours collection of urine. Oral and written instructions on how to collect 24-hour urine were given. Patients were directed to start the 24 h urine collection immediately after discarding their first void of urine in the morning, and to include a final void at completion of the collection period. Between 7 and 9 AM, when patients went to the laboratory to deliver the

collected urine, they were asked to collect (via the midstream technique) a fasting spot urinary sample for UAC. The renal status of the subjects was assessed by the serum creatinine levels. The study group subjects were requested to provide early morning urine sample for spot urine test or a sample (collected anytime between 8 am – 8 pm) and 24 hours urine sample. The urinary albumin was estimated in both the 24 hours and spot urine samples. The two samples were processed immediately and analyzed by two different technicians; the values were unknown to each other.

Urinary microalbumin was determined by the immunoturbidimetric method. Reference value was 0-25 mg/l and range of 0-400 mg/L with a detection limit of 0.7 mg/l. 24-hour urinary albumin excretion was determined by photometric test according to biuret method. SPSS version 12 was used to record and analyze the gathered data. Descriptive statistic and frequencies of the spot urine albumin and 24 hours urinary albumin were measured. The sensitivity, specificity, negative predictive value, positive predictive value, accuracy and efficacy of spot urine microalbumin test was calculated in comparison to 24-hour urine protein test.

Results

The study included 289 patients with DM, who did not have overt proteinuria on routine urine dipstick testing on two occasions. Demographic characteristics of the patients are presented in Table 1.

Age (years) Range (Mean± SD)	22-66(54.06±9.5)
Gender Males; n(%) Females; n(%)	155 (53.63) 134 (46.37)
Type of DM Type 1 DM; n(%) Type 2 DM; n(%)	39 (13.49) 250 (86.50)

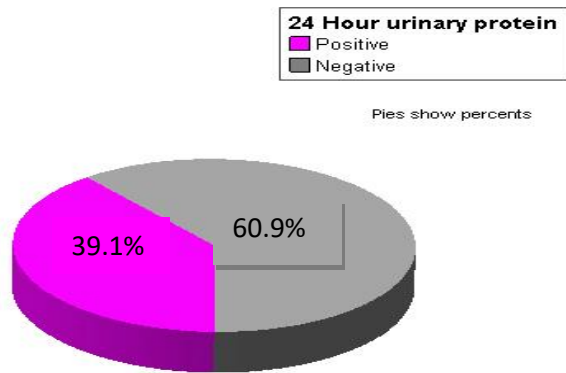


Figure 1: 24 Hour urinary protein

Among 289 patients with diabetes, 113 (39.1%) patients had positive 24-hour urine protein and 176 (60.9%) patients had negative 24-hour urine protein (Figure 1). Total 98 patients (33.9%) had positive spot urine microalbumin test and 191 (66.1%) had a negative spot urine microalbumin test (Figure 2).

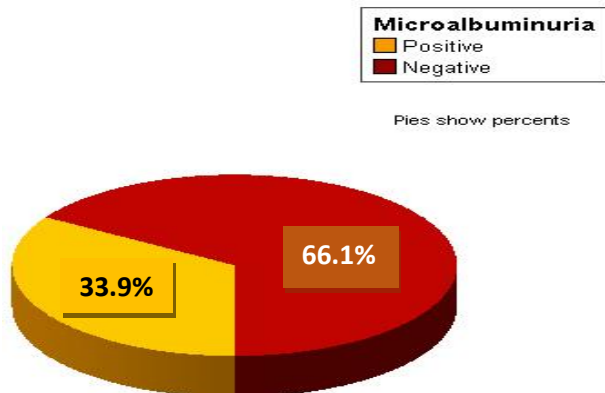


Figure 2: Microalbuminuria

The calculated sensitivity ($TP/TP+FN$) of the spot urine microalbumin test in comparison to 24-hour urine protein test was 83%. The calculated specificity ($TN/TN+FP$) of the spot urine microalbumin test in comparison to 24-hour urine protein test was 97.7%. The calculated positive predictive value ($PPV=TP/TP+FP$) of the spot urine microalbumin test in comparison to 24-hour urine protein test was 96%. The calculated negative predictive value ($NPV=TN/TN+FN$) of the spot urine microalbumin test in comparison to 24-hour urine protein test was 90%. In total spot urinary microalbumin test, accuracy ($TP+TN/TP+TN+FP+FN$) was 92%. When the results of

spot urine microalbumin test and 24-hour urinary protein test were compared using the chi-square test it was found that patients with a positive spot urine microalbumin test had a statistically significant probability of having a confirmed diabetic nephropathy on 24-hour urinary protein test; $p=0.00$ (Table 2).

		24-hour urine protein		p-value
		Positive	Negative	
Spot urine microalbumin	Positive	TP = 94	FP = 4	0.00
	Negative	FN = 19	TN = 172	

Discussion

Measurement of albumin excretion in a 24-h urine collection has long been the “gold standard” for quantitative evaluation of albuminuria in diabetic patients; however, collection errors due to improper timing and missed samples may lead to significant over- and underestimation of albuminuria. For convenience and consistency, the American Diabetes Association and the National Kidney Foundation have recently recommended measurement of albumin-to-creatinine ratio (ACR) in a random spot urine collection for the diagnosis of microalbuminuria. The guidelines recommended using a first-morning sample because of the potentially higher correlation with 24-h albumin excretion, but a random sample is also considered acceptable if a first-morning specimen is not available. The significance of microalbuminuria is that it is the predictor of clinical proteinuria and chronic renal failure in Insulin dependent diabetes mellitus (IDDM) and the early index of cardiovascular morbidity and mortality as well as diabetic nephropathy in non-insulin dependent diabetes mellitus (NIDDM). Therefore, it is important to assess the validity of a random urine sample as a screening test of diabetic nephropathy. Previous studies have also shown that albumin measurements in a random urine sample presented almost perfect accuracy for the screening of micro- and macroalbuminuria in diabetic patients and suggested it as a valid test in screening for diabetic nephropathy.¹⁴ The study clearly shows that the spot microalbuminuria test provides an equivalent result compared to 24 hours in both type 1 and 2 diabetics. Our

study also concludes that spot urine samples can be effectively used to detect microalbuminuria in patients of diabetes mellitus especially in the Pakistani subset of diabetics.

Some patients have microalbuminuria at the time of diagnosis, which may be due to previously undiagnosed diabetes or some other disease that is responsible for the microalbuminuria. Microalbuminuria is associated with declining kidney function, progression to macroalbuminuria, and increased long-term mortality. However, remission to normoalbuminuria may occur. Factors associated with remission include short duration of microalbuminuria, better glycemic control, better blood pressure control, and use of ACE inhibitors or angiotensin receptor blockers.

Diabetic nephropathy is the commonest cause of renal failure in End-Stage Renal Disease [ESRD]. Mortality rates in these patients during dialysis are much higher than they are for non-diabetic cases. Given the worldwide high prevalence of both diabetes and hypertension, and of renal involvement in both disorders, it is important to detect renal disease promptly—through screening for microalbuminuria—when it is still at the reversible stage, in order to reduce both mortality and treatment cost in those affected.

In type 1 diabetes, approximately 20 to 30 percent will have microalbuminuria after a mean duration of diabetes of 15 years.¹⁵ Less than half of these patients will progress to overt nephropathy. Microalbuminuria may regress or remain stable in a substantial proportion, probably related to glycemic and blood pressure control. Various studies have reported that the renal prognosis of type 1 diabetes, including the rate of progression to ESRD, has dramatically improved over the last several decades.¹⁶ The prevalence of progressive renal disease has generally been lower in type 2 diabetes than in type 1 disease. However, current data suggest that the renal risk is equivalent in the two types of diabetes.³ In both types there is regression of microalbuminuria with good glycemic control.¹⁷ Apart from glycemic control various risk factors have been found associated with development of overt nephropathy in these patients. These include genetic susceptibility (more chances of nephropathy in patients having diabetic sibling or parent) age (increased risk with increasing age and increases duration of disease

in type 2 diabetes/ lower risk in patients diagnosed before the age of 5 years in Type 1 diabetes), Obesity (increased BMI related to higher glucose level), hypertension, smoking and use of oral contraceptives etc.¹⁸⁻²¹

Microalbuminuria is now widely recognized as a sign of abnormal vascular function and increased vascular permeability. It has also been considered the first indication of renal injury in patients with diabetes. In addition, accumulating evidence suggests that microalbuminuria is associated with a higher cardiovascular risk as well as with a higher mortality independent of other risk factors. Thus screening for microalbuminuria is currently recommended for all patients with diabetes or kidney disease because it also provides valuable information about their cardiovascular risk profile. In a study conducted on a total of 717 adult diabetic in Diabetes Center in Tokyo, Japan, a strong relationship was found between ACRs measured from first-morning and spot urine samples, yielding a linear correlation on a logarithmic scale.²² Other studies have also proved that convenient and accurate protein:creatinine ratio on random urine samples is a reliable method for estimation and screening of early proteinuria than the quantification by collection of 24 hours urine samples²³⁻²⁵

Conclusion

Spot urine microalbumin test is a highly sensitive and specific tool in the diagnosis of early diabetic nephropathy with high positive predictive value. Spot urine microalbumin test offers the advantage of speed, simplicity and early diagnosis. Spot urine microalbumin test has fairly acceptable accuracy as compared to 24-hour urine protein test.

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