

Original Research Article

FREQUENCY OF SPOT URINE PROTEIN:CREATININE RATIO AND 24-HOUR URINARY PROTEIN IN TYPE 2 DIABETES MELLITUS PATIENTS

ABSTRACT

OBJECTIVE: To determine the frequency of spot urine protein:creatinine ratio and 24-hour urinary protein in type 2 diabetes mellitus patients

MATERIAL AND METHODS: This prospective hospital based clinical study was conducted in the Department of General Medicine, Liaquat University of Medical & Health Sciences, Jamshoro, between the periods of six months from 10st May 2018 to 9th November 2019 through a consecutive sampling technique. All the patients having age more than 18 years of both gender, and type 2 diabetes mellitus were enrolled in this study. Twenty-four hour urine sample was collected into the container having 5mL of 10% thymol in isopropanol as a preservative for 24 hours. A sample of 2mL was also taken for evaluation of proteins. Total volume of urine was noted and calculation was done for 24 hours. The proteinuria ≥ 300 mg/dl in 24-hour urine sample was considered as significant proteinuria. Kappa statistics was used to find agreement between spot urine protein and 24 hours urinary protein.

RESULTS: Total 95 patients were evaluated and their mean age was 41.91 ± 14.29 years, with male predominance (n = 66, 69.4%). Average 24 hour urinary protein was 1216.99 ± 949.51 mg and spot-urinary evaluation of protein was 1919.12 ± 2129.25 mg. The agreement between spot urinary protein creatinine ratio and 24 hour urinary protein was found in 82.1% cases through

Kappa statistics and the calculated agreement between the two procedures was 0.975 which provides sufficient agreement to use spot protein:creatinine ratio in routine to diagnose proteinuria.

CONCLUSION: The study have shown that the protein:creatinine ratio for a random urine sample might be used to rule out the presence of significant proteinuria as defined by a quantitative measure of the 24-h protein excretion.

KEY WORDS: Microalbuminuria, Spot Urine P:C Ratio, 24-h Urinary Protein, Type 2 Diabetes Mellitus

INTRODUCTION:

Diabetes mellitus (DM) is a chronic metabolic disorder and affects almost every organ in a body through its microvascular and macrovascular complications (1). Patients with untreated diabetes mellitus are associated with higher rates of complications that cause significant morbidity and mortality. Which is why, this chronic disease has become a major public health concern and can be very damaging as well as. According to the World Health Organization (WHO), DM affects more than 170 million people worldwide and current prevalence in Pakistan is 11.77%, and this number will rise to 370 million by 2030 (2). In a study conducted by Gross JL has shown that almost 25% of the patients with diabetes mellitus eventually develop some sort of kidney disease (3).

Detection of kidney disease in a patient with diabetes mellitus is quite easy and a simple blood test (urea and creatinine) and a urine test may indicate renal function deterioration and presence of microalbuminuria (which is also the first clinical signs of renal dysfunction in patients with diabetes mellitus), respectively (4). In a study conducted by Chowta NK has observed prevalence of microalbuminuria was 37% (5) while similar prevalence (34%) observed in Karachi Pakistan (6). Microalbuminuria is rarely reversible but may progress to overt proteinuria in around one third of patients with type 2 diabetes mellitus as compare to type 1 diabetes mellitus. As per

definition, microalbuminuria is defined as persistent presence of urine albumin ranging between 30mg/day to 300mg/day is labeled as microalbuminuria.

24 hour urine collection is required to ascertain the protein excretion throughout the day, which is contrary to the statement that concentrated urine is required for determination of urine protein creatinine ratio. Now, it is proven that, urine protein creatinine excretion is constant throughout the day and there is no need to do the collection of 24 hour urine if the kidney glomerular filtration (GFR) rate is steady (7, 8). While, on the other hands, some clinicians also use random collection of urine sample called or “spot” urine sample and some also use urine specific gravity for determination of a ratio (9). Fagerstrom P and colleagues (10) in his study have observed that albumin excretion is much less throughout the day when it was expressed as a ratio to creatinine or urine specific gravity. A number of papers are published on this subject in the western world but there is shortage of the data in Pakistani population. Due to this ambiguity, I would like to carry out this study. The study aims to establish the utility of spot voided urine samples for detection of proteinuria as a credible and time saving method.

PATIENTS AND METHODS:

This prospective hospital based clinical study was conducted in the Department of General Medicine, Liaquat University of Medical & Health Sciences, Jamshoro, between the periods of six months from 10st May 2018 to 9th November 2019 through a consecutive sampling technique. All the patients having age more than 18 years of both gender, and type 2 diabetes mellitus were enrolled in this study after taking their informed consent. Exclusion criteria for this study was; presence of diseases which may cause bias in our study by causing proteinuria i.e. hypertension,

nephrotic syndrome, acute renal failure, impaired renal function due to non-diabetic cause, and hyperlipidemia.

The data was collected on the predesigned proforma, which includes patient demographics, relevant history, and relevant investigations. Twenty-four hour urine sample was collected by instructing the patients or their attendants to collect all urine into the same container having 5mL of 10% thymol in isopropanol as a preservative for 24 hours. A sample of 2mL was taken for evaluation of proteins. Total volume of urine was noted and calculation was done for 24 hours. The proteinuria $\geq 300\text{mg/dl}$ in 24-hour urine sample was considered as significant proteinuria.

A random urine sample of 5mL was collected on next day any time just before the analysis and after completion of 24 hour collection. The fasting blood glucose, glycated hemoglobin, urine creatinine, and urine protein was estimated by kit method using semiautomatic analyzer Hitachi 912. The fasting blood glucose and glycated hemoglobin were estimated to know the diabetic status of the patient. Protein-creatinine ratio was calculated by dividing the urinary protein concentration by urinary creatinine concentration. HbA1c was only done once at the beginning of study.

DATA ANALYSIS PROCEDURE:

Statistical package for social sciences (SPSS) version 21 was used for data analysis purpose. Descriptive statistics included mean \pm standard deviation (SD) was calculated for continuous data, i.e., age, serum creatinine, twenty four hour urinary protein, HbA1c, urinary creatinine and urinary protein. Frequencies were calculated from the categorical data, i.e., gender, microalbuminuria, fasting blood sugar, random blood sugar, and protein:creatinine ratio. Kappa statistics was used to find agreement between spot urine protein and 24 hours urinary protein.

Effect modifiers were controlled through stratification of age, gender, HbA1C and applied chi square test taken $p \leq 0.05$ as significant.

RESULTS:

Total 95 patients were evaluated and their mean age was 41.91 ± 14.29 years, with range of 18-65 years. Among them 66 were males and 29 were female patients. The overall mean Hb1Ac was $6.18 \pm 0.99\%$ with range of 3.40% (4.40 – 7.80%). Table No. 01.

Table No. 02 shows that average 24 hour urinary protein was 1216.99 ± 949.51 mg with minimum 100mg and maximum 2600mg. In spot-urinary evaluation the average protein was 1919.12 ± 2129.25 mg with minimum 84mg and maximum 9450mg and average creatinine was 1616.0mg with minimum 250mg and maximum 4500mg.

The agreement between spot urinary protein creatinine ratio and 24 hour urinary protein was observed and it was found that with respect to clinical analysis the agreement was found with 82.1% cases, also presented in Figure No. 01. The agreement was statistically analyzed through Kappa statistics and the calculated agreement between the two procedures was 0.975 which provides sufficient agreement to use spot protein:creatinine ratio in routine to diagnose proteinuria Table No. 02. It was observed that out of total 78 patients with agreement, 54 (69.2%) patients were male and 24 (30.8%) were female patients. Among 17 non-agreement patients, frequency of male and

female was 12 (70.6%) and 5 (29.4%), respectively. There was no significant association found between agreement with gender using chi-square test.

It was observed within agreement patients that 30 (38.5%) patients were $\leq 6.0\%$ Hb1Ac and 48 (61.5%) patients were $>6.0\%$ Hb1Ac. Among non-agreement patients, 6 (37.9%) patients were $\leq 6.0\%$ Hb1Ac and 11 (64.7%) patients were $>6.0\%$ Hb1Ac. There was no significant association found between agreement with Hb1Ac groups using chi-square test.

DISCUSSION:

Migrants from Indo-Asian who had microalbuminuria are more prone to faster progression of kidney disease as compared to native Europeans but disease prevalence, risk factors associated with microalbuminuria, and clinical conditions associated is still lacking.

Diabetes mellitus is the most common cause of microalbuminuria and usually it occurs in 20% - 40% of patients when disease duration of more than 10 years and then it progresses to proteinuria in around 20% - 50% of the patients after 5-10 years with average reduction in renal functions is around 10-15 ml/min/year. Detection of microalbuminuria is crucial in the management of patients and it also helps in determining the overall prognosis of such patients. Most common methods used for the screening of microalbuminuria in Pakistan are 24 hour urine collection and measurement of albumin to creatinine ratio in random urine sample.

In our study the most common age group was middle age and among them males were more common than females, 69.4% and 30.5%, respectively. In an epidemiological study conducted in Germany has shown different findings and found people with more than 50 years were more common to have microalbuminuria (5). The age difference is not significant but that showed early onset of diabetes mellitus in our population this could be due to multiple factors such as cousin marriages leads to early onset of such kind of disease, presence of hypertension and diabetes mellitus in more common than their study subjects, and poor dietary habits are also linked with increased burden of kidney diseases in our country. Also, some of the authors also observed change in protein excretion from morning to evening and night; this variation could be from 100% to 500%. The proper scientific reason is still not clear but authors have assumed multiple factors could be linked such as increase in water intake, physical activity, and diet (11-13).

More than 82% of the patients in our study found to have agreement between spot urinary protein creatinine ratio and 24 hour urinary protein. Same observation was observed by the Wahbeh and colleagues (14). However, in another study conducted by Lane C et al (7) have observed that agreement between agreement between spot urinary protein creatinine ratio and 24 hour urinary protein is higher at low level but when the 24- hour urine protein excretion extends more than 2.0gm than agreement become suboptimal.

THE LIMITATIONS OF THIS STUDY:

The limitations of the study are: low sample size and the difficulties in collection of 24 hours urine sample in the female patients. Further study is required with large sample size to emphasize the hypothesis.

Study is confined to a single center with targeted only local subjects, so it is not clear whether our findings can be generalized nationally and internationally. We tested only a single urine sample, while confirmed diagnosis of MA requires persistence on at least two out of three consecutive tests. Screening for albuminuria is also recommended in high-risk subjects. Emphasized was on adequacy of urine collection and it was advised appropriate recollection in cases of doubt. Furthermore, we deleted samples containing values of 24 h urine volume of <500 ml/day.

CONCLUSION & RECOMMENDATION:

Considering the significance and proven role of microalbuminuria in the detection of early renal impairment diabetes mellitus, it is recommended that screening for MA should be incorporated into the management with associated risk factors for MA. The risk factors are similar to those reported from other Asian countries. Because of the adverse impact of proteinuria on survival in subjects with type 2 diabetes, screening and intervention programs should be implemented early at the stage of microalbuminuria and risk factors should be treated aggressively.

The study have shown that the protein:creatinine ratio for a random urine sample might be used to rule out the presence of significant proteinuria as

defined by a quantitative measure of the 24-h protein excretion. This test could be the reasonable alternative to the 24-hour urine sample collection for the detection of significant proteinuria in type 2 diabetes mellitus patients. When results above the cutoff value for the protein: creatinine ratio are obtained, a full 24-h collection and quantification are indicated. Further prospective studies will be required in specific patient populations to validate these conclusions. The findings of this study may be helpful in achieving the goals associated with screening for proteinuria in at-risk populations.

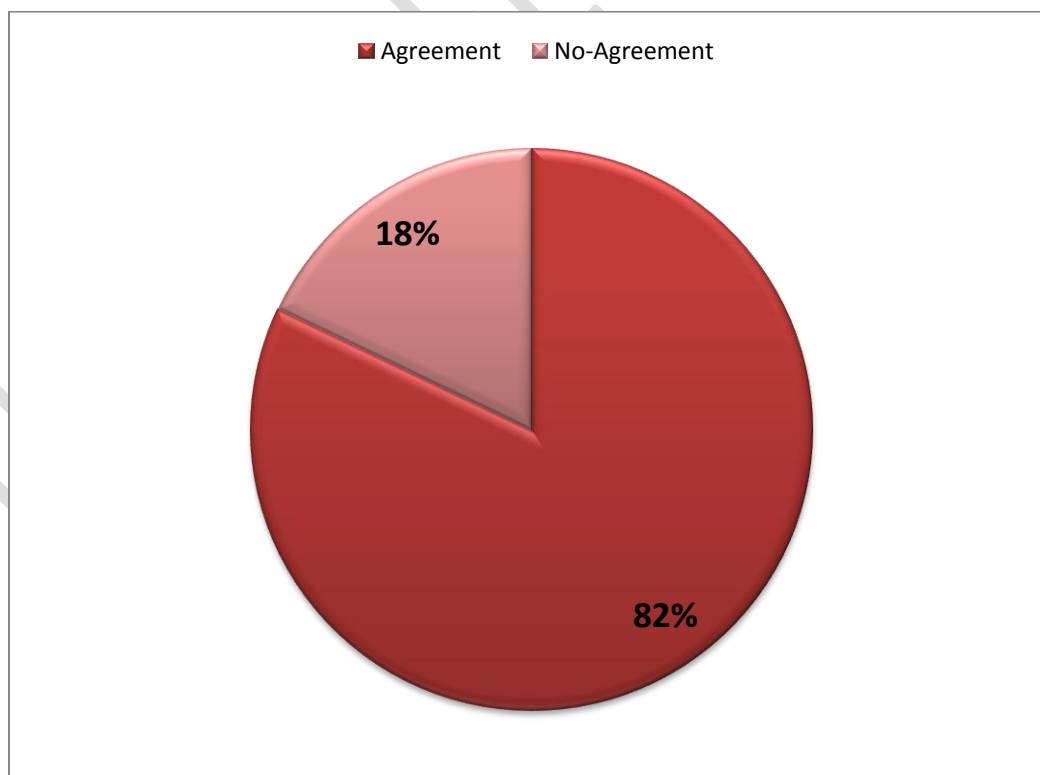
TABLE NO. 01: BASELINE AND CLINICAL CHARACTERISTICS OF STUDY**SUBJECTS****(N = 95)**

Baseline Characteristics	N (%)
	(N = 95)
Age – years	
Mean age \pm SD	41.91 \pm 14.29
Range	
<40	40 (42.1)
>40	55 (57.8)
Gender	
Male	66 (69.4)
Female	29 (30.5)
Marital Status	
Married	62 (65.2)
Single	33 (34.7)
Area of residence	
Urban	68 (71.5)
Rural	27 (28.4)
Clinical Characteristics	
Level of HbA1c - %	
Mean age \pm SD	6.18 \pm 0.99

<6.5	36 (37.8)
>6.5	59 (62.1)
Microalbuminuria	
Yes	30 (31.5)
Macroalbuminuria	
Yes	65 (68.4)
Protein-Creatinine Ratio	
Normal	29 (30.5)

FIGURE NO. 01: PERCENTAGE OF AGREEMENT BETWEEN SPOT PROTEIN-CREATININE RATIO AND 24 HOUR URINARY PROTEIN

(N = 95)



**TABLE NO. 02: DESCRIPTIVE STATISTICS OF 24 HOUR URINARY PROTEIN AND
SPOT URINARY PROTEIN CREATININE RATIO**

(N = 95)

Characteristics	Mean	SD	Range	Minimum	Maximum
24 Hour Urinary Protein	1216.99	949.51	2500	100	2600
Spot Urinary Protein	1919.12	2129.25	9366	84	9450
Spot Urinary Creatinine	1616	1052.73	4250	250	4500

**TABLE NO. 03: KAPPA STATISTICS FOR ANALYSIS OF AGREEMENT BETWEEN
SPOT PROTEIN-CREATININE RATIO AND 24 HOUR URINARY PROTEIN**

(N = 95)

Characteristics		Spot Protein-Creatinine Ratio			
		Normal	%	Not Normal	%
24 Hour Urinary Protein	Normal	29	30.5	1	1.1
	Not Normal	0	0	65	68.4
Kappa value	Total	29 (30.5%)		66 (69.5%)	
		0.975			
p value		<0.001			

REFERENCE LIST

1. Memon MS, Arain ZI, Naz F, Zaki M, Kumar S, Burney AA. Prevalence of type 2 diabetes mellitus in hepatitis C virus infected population: a Southeast Asian study. *J Diabetes Res.* 2013;2013:539361.
2. Meo SA, Zia I, Bukhari IA, Arain SA. Type 2 diabetes mellitus in Pakistan: Current prevalence and future forecast. *J Pak Med Assoc.* 2016;66(12):1637-42.
3. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care.* 2005;28(1):164-76.
4. Afkhami-Ardekani M, Modarresi M, Amirchaghmaghi E. Prevalence of microalbuminuria and its risk factors in type 2 diabetic patients. *Indian J Nephrol.* 2008;18(3):112-7.

5. Chowta NK, Pant P, Chowta MN. Microalbuminuria in diabetes mellitus: Association with age, sex, weight, and creatinine clearance. *Indian J Nephrol.* 2009;19(2):53-6.
6. Ahmedani MY, Hydrie MZ, Iqbal A, Gul A, Mirza WB, Basit A. Prevalence of microalbuminuria in type 2 diabetic patients in Karachi: Pakistan: a multi-center study. *J Pak Med Assoc.* 2005;55(9):382-6.
7. Lane C, Brown M, Dunsmuir W, Kelly J, Mangos G. Can spot urine protein/creatinine ratio replace 24 h urine protein in usual clinical nephrology? *Nephrology (Carlton).* 2006;11(3):245-9.
8. Sandilands EA, Dhaun N, Dear JW, Webb DJ. Measurement of renal function in patients with chronic kidney disease. *Br J Clin Pharmacol.* 2013;76(4):504-15.
9. Segev G, Stafford C, Kirby J, Cowgill LD. Sequential changes in urine production, glomerular filtration rate, and electrolyte excretion after mannitol administration. *J Vet Intern Med.* 2019;33(3):1362-7.
10. Fagerstrom P, Sallsten G, Akerstrom M, Haraldsson B, Barregard L. Urinary albumin excretion in healthy adults: a cross sectional study of 24-hour versus timed overnight samples and impact of GFR and other personal characteristics. *BMC Nephrol.* 2015;16:8.

11. Toblli JE, Bevione P, Di Gennaro F, Madalena L, Cao G, Angerosa M. Understanding the mechanisms of proteinuria: therapeutic implications. *Int J Nephrol.* 2012;2012:546039.
12. Mateus J, Newman R, Sibai BM, Li Q, Barton JR, Combs CA, et al. Massive Urinary Protein Excretion Associated with Greater Neonatal Risk in Preeclampsia. *AJP Rep.* 2017;7(1):e49-e58.
13. Kobayashi S, Amano H, Terawaki H, Ogura M, Kawaguchi Y, Yokoo T. Spot urine protein/creatinine ratio as a reliable estimate of 24-hour proteinuria in patients with immunoglobulin A nephropathy, but not membranous nephropathy. *BMC Nephrol.* 2019;20(1):306.
14. Wahbeh AM, Ewais MH, Elsharif ME. Comparison of 24-hour urinary protein and protein-to-creatinine ratio in the assessment of proteinuria. *Saudi J Kidney Dis Transpl.* 2009;20(3):443-7.