Original Research Article

COMPARING SPOT URINE PROTEIN:CREATININE RATIO 24-HOUR URINARY PROTEIN ESTIMATION IN TYPE 2 DIABETES MELLITUS PATIENTS

ABSTRACT

BACKGROUND & OBJECTIVE: 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. This cost effective test easy to perform even in the absence of advance facilities in a hospital. That is why we planned to conducted this study to assess the comparison between of spot urine protein:creatinine ratio with 24-hour urinary protein in patients with type 2 diabetes mellitus.

MATERIAL AND METHODS: This prospective hospital based clinical study was conducted in the Department of General Medicine, Liaquat University of Medical & Health Sciences, Jamshoro, over a period of six months from 10th 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. Three cc blood was taken to determine the serum creatinine levels and twenty-four hour 2ml urine sample was also collected to determine the urinary protein levels. The proteinuria ≥300mg/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: A 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±949.51mg and spot-urine evaluation of protein was 1919.12±2129.25mg. The agreement between spot urinary protein creatinine ratio and 24 hour urinary protein was found in 82.1% of cases through Kappa statistics and the calculated agreement between the two procedures was 0.975 which provides sufficient agreement to use spot urine protein:creatinine ratio in routine diagnosis of 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-hour urine protein excretion.

KEY WORDS: Spot urine protein:creatinine ratio, 24-hour 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. 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). The 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) (4). In a study conducted by Chowta NK, it was observed that the prevalence of microalbuminuria was 37% (5) while similar prevalence (34%) was 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 compared to type 1 diabetes mellitus. As per definition, microalbuminuria is defined as persistent presence of urine albumin ranging between 30mg/day to 300mg/day (7).

Twenty-four 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 (8, 9). The glomerular filtration rate can easily be

measured by using simple blood test in which urea and creatinine levels are obtained to calculate the person's GFR. That is why most of the clinicians use this method to measure the GFR and creatinine levels at the same time. Some clinicians do not rely on creatinine levels obtained from blood sample and they use 24-hour urine sample to assess urine creatinine and protein levels (10). Fagerstrom P and colleagues (11) 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 such data in Pakistani population. Due to this paucity of data in my region, I would like to carry out this study. The study aims to establish the utility of voided spot 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, over a period of six months from 10th 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 e.g. 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.

Specimen collection

Twenty-four hour urine sample was collected by instructing the patients. The patient's first voided morning urine was discarded. Subsequent urine produced for next 24-hours including the next morning's first voided specimen, was collected in containers that were provided by the laboratory. All urine samples were collected 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 urinary protein measurements. Total volume of urine was noted and calculation was done for 24-hours. The proteinuria ≥300mg/dl in 24-hour urine sample was considered as significant proteinuria.

A random urine sample of 5mL was collected on the next day any time just before the analysis and after completion of 24-hour sample 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 including mean ± 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 ≤ 0.05 as significant.

RESULTS:

Total of 95 patients were evaluated and their mean age was 41.91±14.29 years, with range of 47 (18-65) years. Among them 66 were males and 29 were female patients. The overall mean HbA1C was 6.18±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±949.51mg with a minimum of 100mg and maximum of 2600mg. In spot-urinary evaluation the average protein was 1919.12±2129.25mg with minimum 84mg and maximum 9450mg and the average creatinine was 1616.0mg with a minimum of 250mg and maximum of 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 in 82.1% cases; This was 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 routinely to diagnose proteinuria. Table No. 02 showed that out of the total of 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.

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

Baseline Characteristics	N (%)
	(N = 95)
Age – years	
Mean age ± SD	41.91±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 ± SD	6.18±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)
Abnormal	66 (69.4)

FIGURE NO. 01: PERCENTAGE OF AGREEMENT BETWEEN SPOT PROTEIN-

CREATININE RATIO AND 24-HOUR PROTEINURIA

(N = 95)
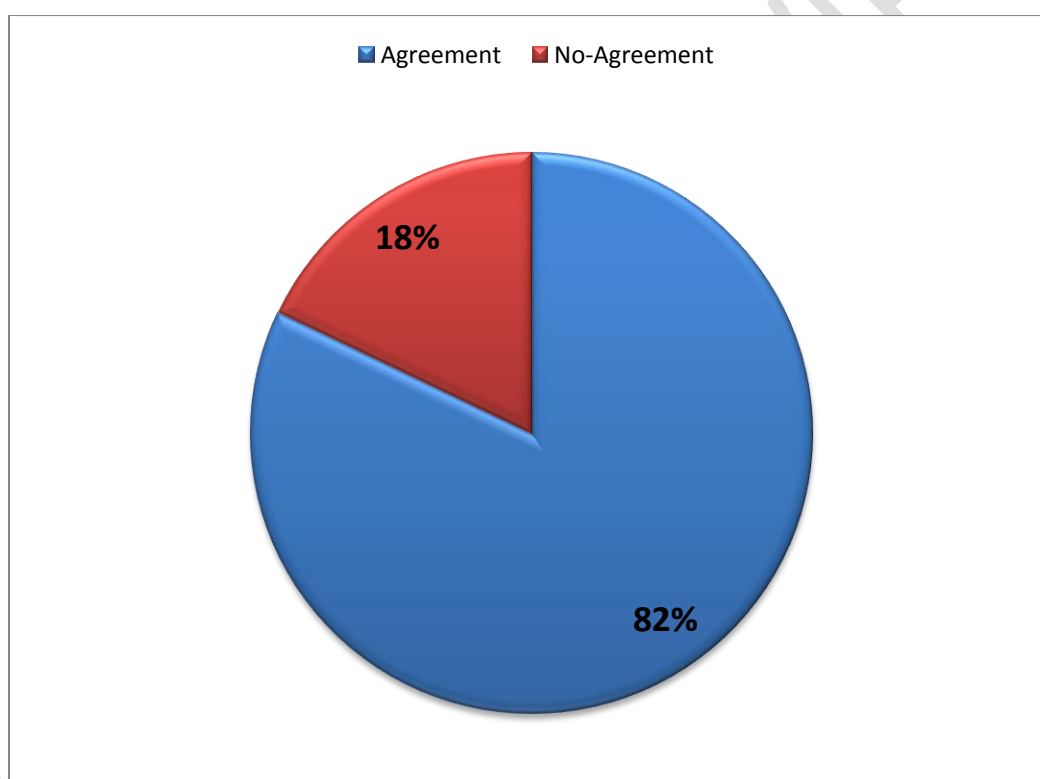
TABLE NO. 02: DESCRIPTIVE STATISTICS OF 24-HOUR URINARY PROTEIN AND SPOT URINARY PROTEIN CREATININE RATIO

(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			

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 associated clinical conditions are still lacking.

Diabetes mellitus is the most common cause of microalbuminuria and usually it occurs in 20% - 40% of patients with 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 of about 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. An epidemiological study conducted in Germany has shown different findings and found that 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, poor dietary habits, and awareness regarding disease (12-14).

More than 82% of the patients in our study were found to have agreement between spot urinary protein creatinine ratio and 24-hour urinary protein. Same observation was noted by Wahbeh and colleagues (15). However, in another study conducted by Lane C et al (8) it was observed that the agreement between spot urinary protein creatinine ratio and 24-hour urinary

protein is higher at lower levels, but when the 24-hour urine protein excretion exceeds more than 2.0gm that agreement become suboptimal.

THE LIMITATIONS OF THIS STUDY:

The limitations of the study are: low sample size and the difficulties in collection of 24-hour 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 which 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. Emphasis was on adequacy of urine collection and appropriate recollection in cases of doubt was observed. Furthermore, we deleted samples containing values of 24-hour urine volume of <500 ml/day.

CONCLUSION & RCOMMENDATION:

Considering the significance and proven role of microalbuminuria in the detection of early renal impairment in diabetes mellitus, it is recommended that screening for MA should be incorporated into the management of DM patients with associated risk factors for MA also, patients with Type 2 diabetes should be screened at diagnosis and yearly thereafter. The risk factors are similar to those reported from other Asian countries (16). 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-hour 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-hour urine 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.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

Consent

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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