

Estimation of Urinary Protein: Creatinine Index in Newly Diagnosed Diabetic Patients



Medical Science

KEYWORDS :

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ABSTRACT

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Proteinuria has been recognized as one of the earliest signs of renal function deterioration in diabetes mellitus. In this study, an attempt has been made to establish the Protein: Creatinine Index (PCI) in random urine samples, as a convenient, quick and reliable method for the estimation of proteinuria in diagnosing and monitoring diabetic nephropathy in newly diagnosed diabetes mellitus patients. Total of 50 samples were collected from the IPD and OPD patients, 25 patients aged 25 to 65 years, who were diagnosed as diabetics and were confirmed by the estimation of fasting serum glucose (>126 mg/dl). 25 normal healthy subjects were age and sex matched with the diabetic patients, selected as the Controls. The comparison of urinary protein, urinary creatinine and urinary protein:creatinine index(PCI) between control and study group was analysed using unpaired "t"-test. The urinary protein levels were significantly increased, urinary creatinine levels were decreased and urinary protein:creatinine index was significantly increased in newly diagnosed diabetics as compared to the non-diabetics(controls). This study suggests that random urine PCI can be a good predictor of significant proteinuria in diabetic nephropathy. This test could be a reasonable alternative to the 24-hour urine sample collection for the detection of significant proteinuria in diabetes mellitus patients.

Introduction:

Diabetes mellitus (DM) is characterized by hyperglycemia over a prolonged period². This high blood sugar produces the symptoms of frequent urination, increased thirst, and increased hunger. Untreated, diabetes can cause many complications. Acute complications include diabetic ketoacidosis and nonketotic hyperosmolar coma. Serious long-term complications include heart disease, stroke, kidney failure, foot ulcers and damage to the eyes.³ Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β -cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Proteinuria has been recognized as one of the earliest signs of renal function deterioration in DM. Proteinuria occurs due to alterations in the glomerular permeability and later, due to a failure in the reabsorption of filtered protein by the tubular cells. Normally, most of the healthy adults excrete 20-150 mg of protein in urine over 24 hours.⁴ In DM, the vascular permeability increases and albuminuria appears when the metabolic regulation is poor, because of glycosylation and a loss of negative charges on the glomerular membrane. Diabetic nephropathy and deterioration of the renal function in Diabetes mellitus are preventable by the diagnosis of proteinuria at an early stage.⁵ In an attempt to fulfill the need for a reliable and quick measurement of the urinary protein, various researchers have proposed the calculation of ratios such as the Urinary Protein/Urinary Creatinine (UP/UC), the Urinary Albumin/Urinary Creatinine (UA/UC) and the PCI on spot urine samples.⁶ These parameters take into account the fact that the creatinine excretion remains fairly constant in the presence of a stable Glomerular Filtration Rate (GFR), thus, eliminating the variations in the urinary protein concentration during the day. Good correlation has been found between the results of proteinuria which were obtained from these parameters and those which are calculated from the 24-hour urine samples.⁷ Proteinuria is conventionally detected by qualitative tests, e.g. the sulfosalicylic acid test, Heller's nitric acid test and the heat coagulation test. But, these tests are not sensitive enough to detect the microproteinuria which is seen

during the initial stages of diabetic nephropathy.⁴ In this study, an attempt has been made to establish the PCI in random urine samples, as a convenient, quick and reliable method for the estimation of proteinuria in diagnosing and monitoring diabetic nephropathy in newly diagnosed diabetes mellitus patients. An attempt has also been made to determine the optimal cut off value of PCI for the prediction of significant proteinuria. This study signifies the role of urinary PCI in detecting even a minor increase in the protein excretion in a random urine specimen.

2. Material and methods:

2.1 Ethical clearance: This study was carried out in the Department of Biochemistry in collaboration with the Department of Medicine NIMS Medical College and Hospital, Shobha Nagar, Jaipur, Rajasthan. The institutional ethical clearance was obtained from Ethical Committee of the college

2.2 Study population:

A total of 50 subjects were enrolled into this study after obtaining informed consent. Among the subjects recruited, 25 were diagnosed with diabetes based on fasting serum glucose (> 126 mg/dl) for at least two occasions. Rest of the subjects were non-diabetics who served as an age matched control group. All the participants were enrolled from out-patient department of Medicine, NIMS Medical College and Hospital. All the relevant demographic data and clinical history were obtained by verifying patient records. Exclusion criteria involved, Surgical patients, Pregnant women, ICU admitted patients, Chronic hypertensive patients, Children, Urinary tract infections, Emotional or physical stress, Strenuous exercise. The patients and the controls were instructed to collect untimed spot urine samples. The urine samples were collected at room temperature, without adding any preservatives. Immediately after their collection, the urine samples were analyzed for protein and creatinine.

2.3 Quantitative determination of total urinary protein: Protein reacts in acid solution with pyrogallol red and molybdate to form a coloured complex. The intensity of the colour formed is

proportional to the protein concentration in the sample.⁸

2.4 Estimation of urinary creatinine: The assay is based upon the reaction of creatinine with the sodium picrate as described by Jaffe. Creatinine reacts with alkaline picrate forming a red complex. The time interval chosen for measurements causes avoids interferences from other serum constituents. The intensity of colour formed is proportional to the creatinine concentration in sample.⁹

2.5 Calculation of the Protein Creatinine Index (PCI):¹⁰ The urinary PCI will be calculated by the following equation:

Statistical analysis:

Statistical analysis was done, using IBM SPSS 20 for Windows software Microsoft Excel 2007 and scientific calculator. Student t-test was applied on the data of the case history taken from the patients from NIMS MEDICAL HOSPITAL and correlation of both the urine albumin and urine creatinine was studied. The normal range of the urinary PCI was calculated from the data which was obtained from the urine samples from normal healthy subjects. The Student's 't' test was used to compare the PCIs of the normal healthy controls and the diabetic patients. The results were expressed as Mean \pm Standard Deviation (SD). The comparison of urinary protein, urinary creatinine and urinary protein:creatinine index (PCI) between control and study group was analyzed using unpaired "t"-test. Statistical significance was considered to be significant at a p value of <0.05.

3. Observations and results:

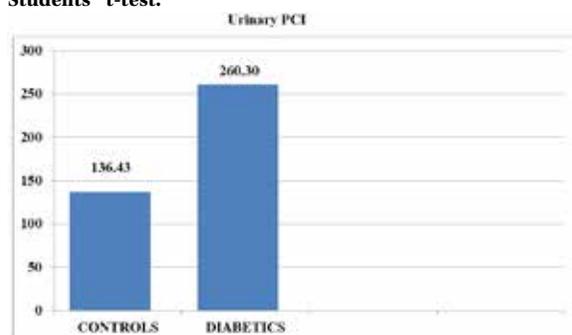
Table 1. Comparison of study variables between control and newly diagnosed diabetic group

Parameter	Control Group (n=25)	Study Group (n=25)	P-value
Urinary Protein (mg/dl)	9.89 \pm 3.92	18.97 \pm 4.90	<0.001
Urinary Creatinine (mmol/dl)	0.82 \pm 0.42	0.73 \pm 0.13	0.2
PCI	136.43 \pm 46.52	260.30 \pm 40.68	<0.001

PCI; Protein:Creatinine Index ,SD; Standard deviation, Values are mean \pm SD

The mean urinary protein concentration which was found in the diabetic group was 18.97 \pm 4.90 mg/dl, and in the control group, it was 9.89 \pm 3.92 mg/dl. The protein excretion in the spot urine samples in the diabetic group was found to be significantly higher in comparison to that in the control group, with a p value of < 0.001.

Fig.1- Comparison of urinary PCI (Protein:Creatinine Index) between control and newly diagnosed diabetic group by Students' t-test.



A significantly higher value of the PCI was observed in diabetic patients (260.30 \pm 40.52) as compared to that in the control group, where the PCI was (136.43 \pm 46.52) (p<0.001). This indi-

cated that the spot urine protein: creatinine index can serve as a baseline predictor of the progression of renal diseases which are secondary to the hyperglycaemia of Diabetes mellitus.

4. Discussion:

In present study, the level of urinary protein, urinary creatinine and protein creatinine index (PCI) among the study group showed significant difference when compared with control group (p<0.05). The mean urinary protein concentration which was found in the diabetic group (newly diagnosed) was (18.97 \pm 4.90 mg/dl), and in the control group, it was (9.89 \pm 3.92 mg/dl). The protein excretion in the spot urine samples in the diabetic group was found to be significantly higher in comparison to that in the control group, with a p value of < 0.001. The amount of creatinine which was excreted in urine in diabetes mellitus patients(newly diagnosed) (0.73 \pm 0.13 mmol/dl) was comparable to that in the control subjects (0.82 \pm 0.42 mmol/dl) ,with p-value 0.2. A significantly higher value of the PCI was observed in diabetic patients(newly diagnosed) (260.30 \pm 40.52) as compared to that in the control group, where the PCI was (136.43 \pm 46.52) (p<0.001).

Biradar et al, reported that the urinary protein excretion was significantly elevated in type 1 and type 2 diabetes mellitus patients. The mean values of the 24-hour urinary protein which were obtained in their study was 1.6 \pm 1.7 gm/day, which correlated well with the P:C ratio of 1.27 \pm 1.55.¹¹ Kumar Anoop et al. studied the amount of creatinine which was excreted in urine in diabetes mellitus patients irrespective of duration of disease(0.70 \pm 0.34 mmol/dl) was comparable to that in the control subjects (0.88 \pm 0.42 mmol/dl) which were concurrent with the results of this study and they also reported that significantly higher value of the PCI was observed in diabetic patients irrespective of the duration of disease (373.04 \pm 98.53) as compared to that in the control group, where the PCI was 114.65 \pm 47.97 (p<0.001).¹²

5. Conclusion:

The present study suggests that random urine PCI can be a good predictor of significant proteinuria in diabetic nephropathy. This test could be a reasonable alternative to the 24-hour urine sample collection for the detection of significant proteinuria in diabetes mellitus patients. It is recommended that the PCI should be specially employed for the assessment for microproteinuria in diabetic patients, when in a few instances a negative result may be obtained by the semi-quantitative dipstick test. The simplicity, accuracy and the lower cost of the PCI justifies its preferential diagnostic use.

REFERENCE

1. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 1997; 20: 1183– 1197.[PMC-NCBI],cited2015.Available from: | 2. Diabetes Fact sheet No.312. WHO. October 2013. Retrieved 25 March 2014. | 3. Kitabchi,Miles,Fisher et al.Hyperglycemic crises in adult patients with diabetes.*Diabetes Care* 32 (7): (Jul 2009) 1335–43. | 4. Marshall WJ, Bangert SK. *Clinical Biochemistry Metabolic and Clinical Aspects*. 2nd Ed. Churchill Livingstone Elsevier L.; 2008. p. 329. | 5. Abuelo JG. Proteinuria: Diagnostic principles and procedures. *Annals of Internal Medicine*.1983;98:186–91. | 6. Keave WF, Eknoyan G. Proteinuria, albuminuria, risk, assessment, detection, elimination (PARADE).A position paper of the National Kidney Foundation. *Am J Kidney Dis*. 1999;33:1004–10. | 7. Matrix HJ, Chi-Yuan HSU. Use of albumin/creatinine ratio to detect microalbuminuria: implications of sex and race. *J Am Soc Nephrol*. 2002;13:1034–39. | 8. Matrix HJ, Chi-Yuan HSU. Use of albumin/creatinine ratio to detect microalbuminuria: implications of sex and race. *J Am Soc Nephrol*. 2002;13:1034–39. | 9. Murray R.L,Kaplan A et al.*Clin Chem the C.V Mosby Co. St. Louis.Toronto.Princeton* 1984;1261-1266 and 418. | 10. Mc Murray JR.In. *Varley's Practical Clinical Biochemistry*. 6th Ed. Butterworth- Heinemann Ltd 2006;436-51. | 11. Biradar SB, Kallaganad GS, Rangappa M, Kashinakurti SV, Retnakaran R. Correlation of spot urine protein-creatinine ratio with 24-hour urinary protein in type 2 diabetes mellitus patients : a cross sectional study *JRMS* 2011.16(5):6334-39. | 12. Kumar Anoop, Kapoor Sandeep and Gupta R.C; *Journal of clinical and diagnostic research*,ISSN-0973-709X; Comparison of Urinary Protein: Creatinine Index and Dipsticks for Detection of Microproteinuria in Diabetes Mellitus Patient; Published online Apr 1, 2013; 7(4): 622–626. |