ORIGINAL RESEARCH

A Cross section study to Correlate Micro Protein and Protein Creatinine Ratio in type 2 Diabetes Mellitus at Rama Medical College Kanpur

[1]Sarfaraz Alam, [2]Dr. Pawan A. Kulkarni, [3]Dr. Shrawan Kumar, [4]Dr. Pavan Kumar Sharma, [5]Dr. Shilpi Singh, [6]Aakash Kumar Shahani

[1]PG Scholar, Department of Biochemistry, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh. India
[2]Professor, Department of Biochemistry, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh. India
[3]Professor, Department of Medicine, Rama Medical College Hospital and Research Centre, Kanpur, U.P. India
[4]Associate Professor, Department of Biochemistry, Rama Medical College Hospital and Research Centre, Kanpur, U.P. India
[5]Assistant Professor, Department of Biochemistry, GSVM Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh. India
[6]Tutor, Department of Biochemistry, Rama Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh. India

Corresponding author
Dr. Shilpi Singh
Assistant Professor, Department of Biochemistry, GSVM Medical College Hospital and Research Centre, Kanpur, Uttar Pradesh, India
Email: shilpisingh678@gmail.com

ABSTRACT
Diabetic nephropathy is the major cause for chronic renal failure (CRF) and proteinuria is an independent risk factor for end stage renal disease. Hence, early identification and quantification of proteinuria is of prime importance in the diagnosis and management. **Introduction:** Diabetes mellitus 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 various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. **Material and methods:** This study will be conducted in department of Biochemistry, Rama Medical College Hospital &Research Centre Kanpur. Study taken from IPD& OPD of medicine and department of Biochemistry Lab Rama Medical College Hospital. **Results:** All parameters- glucose p-value =0.0001, microprotein p-value =less than 0.001, protein p-value=0.001, creatinine p-value=0.005, albumin p-value=0.21, totel protein p-value equal 0.05 Between case study /case control p-value is Statistical significant. **Conclusions:** Purpose of This Study Is To Examine Relationship Between Protein Creatinine Ratio And Microprotein, Compare The Diagnostic Performance In Order To Investigate And Identify Relation Between Them In Type-2 Diabetes Mellitus (DM). The Random Urine P:C Ratio Predicts The Amount Of 24-Hour Urinary Protein Excretion With High Accuracy. Hence It Can Be Used As A Faster Diagnostic Substitute For 24-Hour Urinary Protein Estimation.
Key words: Diabetes, diabetic retinopathy, retinopathy, spot urine protein creatinine ratio, Urine PCR

INTRODUCTION

Diabetes mellitus 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 various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. 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. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action

1146

of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action.

Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia[1]

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome.Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease[2].

Type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In the latter category, a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected[3].

Diabetic nephropathy became the leading cause of chronic dialysis in 1998. Since then, the incidence of this condition has increased, with only a recent plateau. However, diabetic nephropathy continues to account for a large proportion of all cases of chronic kidney disease (CKD), and remains by far the most common underlying cause of chronic dialysis among all kidney diseases, consequently leading to the escalation of healthcare costs, thus representing a compelling medico-social issue of interest[4].

Accurate identification and quantification of proteinuria are core elements in the diagnosis and management of chronic kidney disease (CKD). Proteinuria is associated with an increased risk of progressive kidney failure , cardiovascular disease and death , and is used to monitor the progress of kidney disease, or response to therapy. Timed urine collections (usually performed over 24 h) are considered the gold standard for quantification of proteinuria but have major limitations. Measurement of total protein:creatinine ratio (TPCR) or albumin:creatinine ratio (ACR) on random ('spot') urine samples correlates well with 24-h total protein and albumin excretion, respectively . Guidelines recommend spot urine samples in preference to 24-hours collections , though some disagree, particularly in the monitoring of glomerular disease [5]

Some guidelines recommend using ACR for all patients with CKD , while others recommend restricting ACR to patients with diabetes mellitus, and using TPCR for all others . Our biochemistry laboratory routinely analyses urine for both ACR and TPCR[6].

Group of proteins that perturb the formation of functional protein dimers by forming non-functional, homotypic protein complexes with their targets, which they regulate in a dominant-negative manner We refer to these protein species as 'microProteins' (miPs)—although some are not small—because the results of their actions are analogous to microRNAs (miRNAs), which are negative regulators of mRNAs. miPs can potentially act in the context of any protein that needs to form functional dimers inorder to perform its function. This review considers several such cases, many of which are transcription factors. In eukaryotes, protein-encoding genes are transcribed by a protein complex containing the enzyme RNA polymerase II. In conjunction with the basal transcription machinery, the rate of transcription is fine-tuned with the help of several transcriptional regulators—also called transcription factors—which act as DNA-binding factors that can either enhance or repress the transcription of a gene. A common feature of transcriptional regulators—which is often a prerequisite for DNA binding—is that they form functional protein homodimer[7]

Purpose of this study is to examine relationship between protein creatinine ratio and microprotein, compare the diagnostic performance in order to investigate and identify relation between them in type-2 Diabetes Mellitus (DM).

MATERIAL AND METHODS

Study Setting – This study will be conducted in department of Biochemistry, Rama Medical College Hospital &Research Centre Kanpur. Study taken from IPD& OPD of medicine and department of Biochemistry Lab Rama Medical College Hospital.

Study Subjects - 50 subjects with known history diabetes will be admitted at Rama Medical college hospital. **50** subjects Age, sex and gender matched healthy control will be included in the study.

Study Design – Case control study.

Study Period – This study will be conducted from July 2022 to June 2023.

Specimen collection: About 5 ml of fresh random urine sample will be collected in disposable container and serum sample in plain vial under all aseptic precaution after explaining the procedure to the study subjects. Sample preserved at -20ºC until analysis.

Inclusion Criteria

a. Patient with known case of diabetes mellitus-2(control & uncontrolled)disease

b. People & individuals who went to OPD for routine health checkup as controls.

Exclusion Criteria

a. Patients with endocrinological disorders.

b. Patients with liver disorder, pregnant women.
c. Also acutely ill patients, patients on statins.
d. Participants with myeloproliferative disorders and in therapy with. cytotoxic drugs, pregnant women ,lactating mother's ,renal or hepatic disorders
e. Newly diagnosed renal disease patients

Statistical Analysis: Appropriate Statistical test will be applied to analyze the data.

Ethical Clearance: Ethical clearance will be taken from ethical committee of Rama Medical College Hospital andResearch Centre.

Methodology

Microprotein

Microprotein is estimated by pyrogallol red method on semi Autoanalyser ErbaChem 07[8]

Serum Creatinine

Creatinine is estimated by Jaffe's method or alkaline Picrate method on semiAutoanalyser Erba Chem.[9]
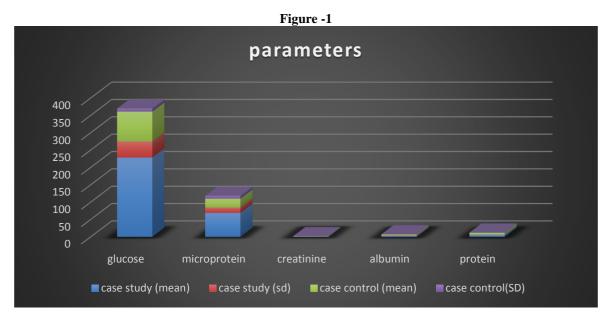
Serum Albumin

Albumin is estimated by Bromo Cresol Green method semi Autoanalyser ErbaChem 07[10]

Reference Ranges

Urine (random) microprotein-1-35 mg/dl [11]
Creatinine – Male -0.7 to 1.4 mg/dl [12]
Female- 0.6 to 1.2 mg/dlAlbumin – 3.5 to 5.2 g/dl [13]
Urine   Albumin   Creatinine   ratio– PROTEIN/CREATININE *1000

RESULTS

Table -1

Parameters	Case study (Mean)	Case study (SD)	Case control (Mean)	Case control (SD)
Glucose	228.4	46.53	86.04	8.98
Microprotein	69.14	15.5	25.46	8.43
Creatinine	0.954	0.35	0.78	0.23
Albumin	3.06	0.62	3.22	0.64
Protein	5.71	0.54	5.43	0.86

Figure -1

Table-2

Parameters	P – (value)	t – (value)
Case Study (Glucose)	Less than 0.0001	21.24
Case Control (Glucose)		
Case Study (Microprotein)	Less than 0.001	17.45
Case Control (Microprotein)		
Case Study (Creatinine)	0.005	2.8
Case Control (Creatinine)		
Case Study (Albumin)	0.21	1.25
Case Control (Albumin)		
Case Study (Protein)	0.05 (Equal)	1.9
Case Control (Protein)		

DISCUSSION

The present study showed a positive correlation between the random urine P: C ratio and 24-hour urinary protein in type 2 diabetes mellitus patients. Yadav BK et al have also shown the similar results[14]. However, the urinary protein excretion over a 24-hour period is the gold standard method. Most of the times, it appears impracticable, especially in outpatient department and female patients to collect 24-hour urine. The high degree of variation in the urinary protein concentration during the day can be attributed to variation in intake of water, rate of diuresis, exercise, recumbency and di-et[15.] Proteinuria is a cardinal manifestation of glomerular or tubular disease that requires meticulously timed (usually 24 hours) urine collection for its evaluation. Complete collection can be assured by indwelling Foley's catheter or extreme awareness of the completeness of urine collection. The non-enzymatic glycation of proteins is also influenced by hyperglycemia, which bind to collagen and proteins that constitute glomerular basement membrane and make glomerular barrier more permeable to proteins[16]. The independent risk factor such as hypertension, together with intrinsic renal toxicity caused due to proteins filtered through glomerular vessels, may contribute to the progression of renal damage[17].Ruggenenti P et al [17] showed that P:C ratio predicted the rate of decline even more accurately than 24-hour urinary protein excretion, which suggests that the random urine P:C ratio is more accurate index of kidney traffic of plasma proteins compared to 24-hour urinary total proteins. The spot morning urine P:C ratio is independent of errors and it is also very minimally affected by wide variations in urinary protein excretion rate which is associated with changes in posture,physical activity and hemodynamic factors. Significance of P:C ratio was shown in various conditions like type-1 diabetes mellitus, non diabetic renal failure, pregnancy, preeclampsia[17], renal transplantation, lupus nephritis etc. Seyed-Ali and Navin Jaipaul [16] found that the random urine P:C ratio is a reliable and practical way of estimating and following proteinuria, but its precision and accuracy may be affected by the level of patient physical activity.Jiunn–Min Wang et al concluded that P:C ratio or albumin to Creatinine ratio, which was obtained by dipstick, can be used to monitor the potential risk of renal diseases in outpatients, hypertensive and diabetic patients. The limitations of the present study were the low sample size, and the difficulties in collection of 24 hours urine sample in the female patients. Thus, early detection and treatment are helpful in improving the quality of life and prevention of blindness in diabetics.

CONCLUSION

In conclusion, the present study suggests that a random urine P:C ratio predicts the amount of 24-hour urine protein excretion with high accuracy. 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. Hence P: C ratio serves as an early, accurate, convenient, inexpensive and reliable tool to estimate proteinuria.

REFERENCES

1. The 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 20:1183–1197, 1997
2. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 26:3160– 3167, 2003
3. Carpenter MW, Coustan DR: Criteria for screening tests for gestational diabetes. Am J ObstetGynecol 144:768–773, 1982.
4. Committee for Statistical Surveys, Japanese Society for Dialysis Therapy (JSDT). Current state of dialysis therapy in Japan, 2013 illustrated. Available at: http://docs.jsdt.or.jp/ overview/index.html.
5. Scottish Intercollegiate Guidelines Network. Diagnosis and Management of Chronic Kidney Disease. Edinburgh: SIGN, 2008 13.
6. Caring for Australasians with renal impairment (CARI). 2003. Available from: www.cari.org.au (10 February 2010, date last accessed).
7. Alvarez JP, Pekker I, Goldshmidt A, Blum E, Amsellem Z, Eshed Y (2006) Endogenous and synthetic microRNAs stimulate simultaneous, efficient, and localized regulation of multiple targets in diverse species. Plant Cell 18: 1134–1151
8. Watanabe, N., Makino, K., Kameri, S., Okubo, A., Yamanaka, M. and Osawa, S.Microprotein.: The Journal of Clinical Pathology, 32 suppl. 227 (1984).
9. Labbé D, Vassault A, Cherruau B, Baltassat P, Serum Creatinine, Bonète R, Carroger G, Costantini Guérin S, Houot O,Lacour B, Nicolas A, Thioulouse E, Trépo D.Ann Biol Clin (Paris). 1996;54(8-9):285-98.
10. B T Doumas, W A Watson, Serum Albumin. H G Biggs PMID: 5544065 DOI: 10.1016/0009-9981(71)90365-2
11. Watanabe ,N ,et,al, (1986),Urine Random Microprotein. Clin, Chem, 32; 1551.
12. Slot,C. (1965) Scand. Creatinine, J.Clin Lab.Invest 17,381.
13. Chernecky CC, Berger BJ. Albumin - serum, ,. In: Chernecky CC, Berger BJ, eds. Laboratory Tests and Diagnostic Procedures. 6th ed. St Louis, MO: Elsevier Saunders; 2013:110-112.
14. Rodby RA, Rohde RD, Sharon Z, Pohl MA, Bain RP, Lewis EJ. The urine protein to creatinine ratio as a predictor of 24-hour urine protein excretion in type 1 diabetic patients with nephropathy. The Collaborative Study Group. Am J Kidney Dis 1995; 26(6): 904-9.
15. .Price CP, Newall RG, Boyd JC. Use of protein:creatinine ratio measurements on random urine samples for prediction of significant proteinuria: a systematic review. Clin Chem 2005; 51(9): 1577-86.
16. Sadjadi SA, Jaipaul N. Correlation of random urine protein creatinine (P-C) ratio with 24-hour urine protein and P-C ratio, based on physical activity: a pilot study. Therapeutics and Clinical Risk Management 2010; 6(351): 7.
17. Min Wang J, Yu-Lin C, An Tsai F, Yi Chen J, Chen Koa Y. Test dipstick for determination of urinary protein, creatinine and protein / creatinine ratio. J Biomed Lab Sci 2009; 21(1): 23-8.