International Journal of Biochemistry Research & Review
11(3): 1-6, 2016, Article no.IJBCRR.24760
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

Cystatin C- An Early Diagnostic Biomarker of Diabetic Kidney Disease

Sudarshan N. Shelke[1*], Pramod W. Ingale[1], Pooja S. K. Rai[1] and Shimi Sundharan[1]

[1]Department of Biochemistry, LTM Medical College, Sion, Mumbai, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SNS designed the study and wrote the protocol. Author PWI supervised the work. Authors SNS and SS carried out all laboratories work and performed the statistical analysis. Authors PWI and PSKR managed the analyses of the study. Authors SNS and PSKR wrote the first draft of the manuscript. Author SNS managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2016/24760
Editor(s):
(1) Dileep G. Nair, Ministry of Higher Education, Sultanate of Oman, Oman.
Reviewers:
(1) Brijesh Mukherjee, Sambalpur University, India.
(2) Rupali Sengupta, SNDT Women's University, India.
(3) Anonymous, Cairo University, Egypt.
(4) Tinei Shamu, Newlands Clinic, Harare, Zimbabwe.
Complete Peer review History: http://sciencedomain.org/review-history/13909

Original Research Article

Received 31st January 2016
Accepted 19th March 2016
Published 29th March 2016

ABSTRACT

Aim: This study aimed to evaluate serum cystatin C vs. Serum creatinine and BUN as a potential better marker for early diagnosis of diabetic kidney disease.
Study Design: Cross-sectional study.
Place and Duration of Study: Department of Biochemistry, LTM Medical College, Sion, Mumbai, between January 2014 and November 2014.
Methodology: Diagnosed patients of type 2 diabetes mellitus were evaluated based on detailed history, clinical examination and laboratory investigations. Patients having frank proteinuria were excluded. Patients without proteinuria were tested for microalbuminuria. In study group, we included 50 patients having microalbuminuria and in control group, 50 patients without microalbuminuria. Blood urea nitrogen (BUN), serum creatinine and serum cystatin C were estimated. Results were

**Corresponding author: E-mail: drsudarshanjs@gmail.com;*

analysed by unpaired t-test & P-value was determined.
Results: Serum cystatin C was found to be increased in the study group as compared with controls and the difference was statistically significant (*P = .000*). BUN (*P = .26*) and serum Creatinine (*P = .11*) were found to be slightly higher in cases compared to control group but values were within reference range.
Conclusion: The results of this study suggest that serum cystatin C measurement is a useful, practical tool for the evaluation of renal involvement in the course of diabetes. BUN and serum creatinine values are affected by many factors like age, sex, muscle mass, diet. Serum cystatin C estimation offers a hope that diabetic kidney disease can be well prevented with appropriate interventions.

Keywords: Diabetic kidney disease; microalbuminuria; serum cystatin C; serum creatinine.

1. INTRODUCTION

Diabetes is one of the most challenging health problems of the 21st century. The prevalence of diabetes is increasing globally. Type 2 Diabetes Mellitus (Type 2 DM) constitutes about 85% to 95% of total diabetic cases in developed countries and accounts for higher percentage in developing countries [1]. Moreover, type 2 DM is a significant cause of premature mortality and morbidity related to cardiovascular disease, macrovascular complications and microvascular complications in older adults [2-4].

Diabetes leads to various chronic complications which can be divided into vascular and nonvascular complications. Diabetes has become the most common single cause of end stage renal disease (ESRD) worldwide [5]. About 20-30% of patients with type 1 or type 2 DM develop evidence of nephropathy, but in type 2 DM, a considerably smaller fraction of these progresses to ESRD. However, because of much higher prevalence of type 2 DM, these patients constitute over half of patients with nephropathy needing dialysis [6]. The diabetic kidney disease progresses from appearance of low but abnormal levels of (≥ 30 mg to 299 mg/day) albumin in urine (stage of microalbuminuria) to stage of macroalbuminuria/clinical albuminuria (≥ 300 mg/day) to ESRD. Progress from microalbuminuria to macroalbuminuria usually takes 10-15 years. It remains under-diagnosed in the diabetic population. Type 2 DM may be present for decades before diagnosis and patient may present with complications at the time of diagnosis [7]. So, early recognition of diabetic kidney disease by health care professionals is vital for proper management.

Although serum creatinine has been widely used as a marker, it is not able to pick up early decreased renal function. Therefore, various plasma low molecular weight proteins have been suggested as valuable markers of decreased renal function in place of serum creatinine.

Although many investigations are available, many of these are not done in public hospitals, so diagnosis of diabetic kidney disease at early stage is missed. This could be due to non availability of resources, manpower or funds. This study aimed to evaluate serum cystatin C vs. Serum creatinine and BUN as a potential better marker for early diagnosis of diabetic kidney disease.

2. MATERIALS AND METHODS

The study was conducted in LTM Medical College, Sion, Mumbai after the approval from institutional ethics committee. The diagnosed patients of type 2 DM attending medicine OPD at LTM Medical College, Sion, Mumbai were enrolled for the study.

Patients having frank proteinuria (≥ 300 mg/day), any known renal disease, taking any medications affecting renal functions (captopril, ciprofloxacin, aspirin, omeprazole, phenytoin etc.), patients with urinary tract infections or hematuria or febrile illness, patients having paraplegia, muscle wasting disease or any neuromuscular disorders were excluded.

On fulfilling the criteria, the study design was explained to the patients with the help of information sheet. Then the written consent was taken.

A twenty four hrs urine sample was tested for proteinuria by using Trichloroacetic acid (TCA) method. Patients having frank proteinuria (≥ 300 mg/day) were excluded. Patients negative for frank proteinuria were further tested for microalbuminuria. In the study group, we

included 50 patients having microalbuminuria and in the control group, 50 patients without microalbuminuria.

BUN by Urease-GLDH method [8], urine albumin by immunoturbidimetric method [9,10], serum creatinine by modified Jaffe's method [11] and serum cystatin C by immunoturbidimetry [12] were estimated.

2.1 Statistical Analysis

For each parameter to be studied, mean and standard deviation was calculated. The difference between the study group and control group was measured by Student's unpaired 't' test. P Value less than .01 was considered as statistically significant.

3. RESULTS AND DISCUSSION

Table 1 shows an insignificantly ($p = .26$) increased BUN levels in the diabetic kidney disease patients (10.54±2.38 mg/dl) as compared to the controls (10.06±1.80 mg/dl). We also observed an insignificant ($p = .11$) increased levels of serum creatinine in the diabetic kidney disease patients (0.77±0.13 mg/dl) as compared to the controls (0.74±0.08 mg/dl).

There was a significant ($p < .01$) increase in levels of serum cystatin C in diabetic kidney disease patients (1.61±0.27 mg/L) as compared to the controls (0.85±0.12 mg/L).

4. DISCUSSION

Overt diabetic nephropathy classically begins with dipstick-positive proteinuria (urinary albumin excretion > 300 mg/day) followed or accompanied by the development of hypertension and/or falling glomerular filtration rate (GFR) [13,14]. At this stage, renal lesions of diabetes, including diffuse mesangial expansion and consequent loss of filtration surface, arteriolar hyalinosis, and global glomerular sclerosis are advanced, and the progression towards end stage renal disease may be slowed but not reversed [15].

Overt nephropathy is preceded by a variable period, which is called as 'incipient nephropathy' [14]. This phase of the natural history of diabetic nephropathy has been defined as an elevation of urinary albumin excretion to levels considered predictive of nephropathy risk (30-300 mg/day) but less than the levels that usually give positive dipstick results. The lower limit of urinary albumin excretion, which is said to be predictive of the later development of overt nephropathy, has been variably reported as around 30 mg/day [16-19].

Some patients with microalbuminuria may also have hypertension or a falling GFR, and this group of patients with microalbuminuria more regularly have advanced glomerular lesions [20]. Before these stages, there are many silent years during which no clinical or laboratory parameter is indicative of underlying renal structural lesions or of eventual nephropathy risk. These years are characterized by urinary albumin excretion and normal or elevated GFR. Glomerular structure during the silent period can range from normal to lesions bordering on those of overt nephropathy.

It is likely that microalbuminuria predicts the later development of overt diabetic nephropathy by serving as a functional indicator of underlying renal structural changes. Several studies have shown that slight increase in albuminuria (microalbuminuria) precede the development of clinical diabetic nephropathy by several years [21]. The concept of a prognostic role for microalbuminuria has been extended to suggest that normoalbuminuria ensures no reduction of renal function [22]. Based on this, we divided type 2 DM patients into 2 groups depending on their different degrees of kidney damage (normoalbuminuria and microalbuminuria).

In this study, we aimed at evaluating the different biomarkers for early diagnosis of diabetic kidney disease like BUN, S. Creatinine and S. Cystatin C in a small cohort of patients with type 2 DM by categorizing them into 2 groups based on their urine albumin status. Our data agrees with the studies carried out by Yun Kyung Jeon et al. [23]. The difference was insignificant for serum creatinine, though the mean value was slightly increased in study group.

Out of 50 patients of study group, the increase in serum cystatin C was observed in 47 patients and maximum value was 2.05 mg/L in study group. Our results are in accordance with the studies carried out by Bruce A. Perkins et al. [24], Min Zhang et al. [25], Laura Pucci et al. [26] Jan Kyhse-Andersen et al. [27] and Radovan Hojs et al. [28].

The routine classical evaluation of diabetic kidney disease includes appearance of microalbuminuria, decreased creatinine

Table 1. Comparison of BUN, serum creatinine and serum cystatin C in type 2 DM patients with microalbuminuria (cases) and without microalbuminuria (controls)

Parameter	Controls (n=50)	Cases (n=50)	*P value*
Bun (mg/dl)	10.06±1.80	10.54±2.38	.26
Serum creatinine (mg/dl)	0.74±0.08	0.77±0.13	.11
Serum cystatin C (mg/L)	0.85±0.12	1.61±0.27	.000

clearance and increased serum creatinine. Many clinicians use serum creatinine in evaluating such patients. However, serum creatinine depends on creatinine production, extrarenal elimination and tubular handling [29].

Moreover, tubular involvement may precede glomerular involvement because several tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and a rise in serum creatinine. Therefore, other biomarkers for estimation of renal function have been searched for and one of them was serum cystatin C [30]. Our study results confirmed that cystain C could be one of the additional tubular factors which represents kidney state of diabetic patients.

It was thought that increment in serum Cystatin C was probably due to the tubular phase before glomerular manifestation. This suggests that the serum cystatin C levels are related to subclinical tubular impairment and can be an earlier measurable marker of renal involvement [30]. Our results indicate that serum cystatin C is a novel and reliable marker of GFR. Zahran et al reviewed a number of studies to compare the diagnostic accuracy of serum cystatin C levels and serum creatinine levels in many clinical situations, including transplant patients, patients with native kidney disease, adults as well as paediatric patients. They found that serum Cystatin C was superior to serum creatinine [31].

Until now, traditional measures for assessing renal function such as measuring serum creatinine have been widely used, although they have some limitations. Here, we evaluated the diagnostic efficacy of using serum cystatin C levels and compared these results to those obtained using traditional renal function indicator such as BUN and serum creatinine. Our study found that the diagnostic accuracy of serum cystatin C levels was superior to that of BUN and serum creatinine.

Although serum creatinine has become the most popularly used serum marker of renal function, serum creatinine may be unreliable because it is frequently affected by muscle mass, age, gender, aberrant renal tubular regulation and methodology of determining serum creatinine level. Using serum cystatin C has some advantages over serum creatinine, in that serum Cystatin C level is independent of age, gender, muscle mass, diet, inflammatory status and renal tubular secretion [32]. So serum cystatin C can be considered an ideal indicator of renal function.

5. CONCLUSION

In conclusion, the results of this study suggest that serum cystatin C measurement is a useful, practical tool for the evaluation of renal involvement in the course of diabetes. Though serum creatinine levels are used to assess kidney function, creatinine values are affected by many factors like age, sex, muscle mass, diet etc. Further investigations with a larger sample size and a prospective design are required to confirm the potential application of cystatin C as a useful biomarker for the early detection of diabetic kidney disease.

Diabetic kidney disease is the leading cause of chronic kidney disease and ESRD. Early recognition is vital for further management. Serum cystatin C can be used as an early independent marker of diabetic kidney disease, as routine biochemical parameters like BUN & serum creatinine fail to recognize early diabetic kidney disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. IDF diabetes atlas. International Diabetes Federation; 2009. 4th edition.
2. Huang ES, Liu JY, Moffet HH, John PM, Karter AJ. Glycemic control, complications, and death in older diabetic patients the diabetes and aging study. Diabetes Care. 2011;34(6):1329-1336.
3. De Boer IH, Katz R, Cao JJ, Fried LF, Kestenbaum B, Mukamal K, et al. Cystatin

C, albuminuria, and mortality among older adults with diabetes. Diabetes Care. 2009;32(10):1833-1838.
4. Andrew Moran, Walter Palmas, Lesley Field, Jyoti Bhattarai, Joseph E. Schwartz, Ruth, et al. Cardiovascular autonomic neuropathy is associated with microalbuminuria in older patients With Type 2 Diabetes. Diabetes Care. 2004; 27:972-977.
5. Powers AC. Diabetes mellitus. Faucy As, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J, editors. Harrison's principles of internal medicine 18th edition, chapter 344, 2968-3003.
6. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2004;27(Suppl.1):S5-S10.
7. American Diabetes Association. Nephropathy in Diabetes. Diabetes Care. 2004;27(Suppl.1):S79-S83.
8. Talke H, Schubert GE, Klin. Wochenschr. Enzymatic urea determination in the blood and serum in the warburg optical test. 1965;43:174.
9. Mount J. Clin J. Pathology. 1986;22:12.
10. Schmidtz A, et al. Diabetic Medicine. 1988;5:126-134.
11. Henry RJ, et al. Clinical chemistry-principles and techniques. Harper & Row, II Ed; 1974.
12. Filler G, Bokenkamp A, Hofmann W. Cystatin C as a marker of GFR-history, indication and future research. Clin Biochem. 2005;38:1-8.
13. Tuttle KR, Stein JH, DeFronzo RA. The natural history of diabetic nephropathy. Semin Neph. 1990;10:184-93.
14. Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease: With emphasis on the stage of incipient diabetic nephropathy. Diabetes. 1983; 32(Suppl. 2)64-78.
15. Mauer SM, Steffes MW, Ellis EN, Sutherland DER, Brown DM, Goetz F. Structural-functional relationships in diabetic nephropathy. J Clin Invest. 1984; 74:1143-55.
16. Mogensen C, Christensen C. Predicting diabetic nephropathy in insulin-dependent patients. N EnglJ /Wed. 1984;311:89-93.
17. Viberti G, Jarrett R, Mahmud U, Hill R, Argyropoulos A, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin dependent diabetes mellitus. Lancet. 1982;1:1430-32.
18. Parving HH, Oxenboll B, Svendsen P, Christiansen JS, Andersen A. Early detection of patients at risk of developing diabetic nephropathy: A longitudinal study of urinary albumin excretion. Acta Endocr. 1982;100:550-55.
19. Mathiesen E, Oxenboll B, Johansen K, Svendsen P, Deckert T. Incipient nephropathy in type 1 (insulin-dependent) diabetes. Diabetologia. 1984;26:406-10.
20. Chavers BM, Bilous RW, Ellis EN, Steffes MW, Mauer SM. Glomerular lesions and urinary albumin excretion in type I diabetes without overt proteinuria. N Engl J Med. 1989;320:966-70.
21. Mogensen CE, Hansen KW, Sommer S, Klebe J, et al. Microalbuminuria: Studies in diabetes, essential hypertension, and renal diseases as compared with the background population. Adv Nephrol. 1991;20:191-228.
22. Hansen KW, Mau Pedersen M, Christensen CK, Schmitz A, Christiansen JS, Mogensen CE. Normoalbuminuria ensures no reduction of renal function in type I (insulin-dependent) diabetic patients. J Intern Med. 1992;232:161-167.
23. Yun Kyung Jeon, Mi Ra Kim, Jung Eun Huh, Ji Young Mok, et al. Cystatin C as an early biomarker of nephropathy in patients with type 2 diabetes. J Korean Med Sci. 2011;26:258-263.
24. Bruce A. Perkins, Robert G. Nelson, Betsy EP. Ostrander et al. Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: Results of a 4-Year Follow-Up Study J Am Soc Nephrol. 2005;16: 1404-1412.
25. Min Zhang, Xueying Cao, Guangyan Cai et al. Clinical evaluation of serum cystatin C and creatinine in patients with chronic kidney disease: A meta-analysis. Journal of International Medical Research. 2013;41(4) 944–955.
26. Laura Pucci, Stefano Triscornia, Daniela Lucchesi, et al. Cystatin C and estimates of renal function. Searching for a Better Measure of Kidney Function in Diabetic Patients Clinical Chemistry. 2007;53:3 480-488.
27. Jan Kyhse-Andersen, Camilla Schmidt, Gunnar Nordin, et al. Serum Cystatin C, Determined by a Rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for

glomerular filtration rate CLIN. CHEM. 1994;40/10:1921-1926.
28. Radovan Hojs, Sebastjan Bevc, Robert Ekart, Maksimiljan Gorenjak, Ludvik Puklavec. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function Nephrol Dial Transplant. 2006;21:1855-1862.
29. Stevens LA, Levey AS. Measurement of kidney function. Med Clin North Am. 2005;89:457-73.
30. Choe JY, Park SH, Kim SK. Serum Cystatin C is a potential endogenous marker for the estimation of renal function in male gout patients with renal impairment. J Korean Med Sci. 2010; 25:42-8.
31. Zahran A, El-Husseini A, Shoker A. Can cystatin C replace creatinine to estimate glomerular filtration rate? A literature review. Am J Nephrol. 2007;27:197-205.
32. Mojiminiyi OA, Abdella N. Evaluation of cystatin C and beta-2 microglobulin as markers of renal function in patients with type 2 diabetes mellitus. J Diabetes Complications. 2003;17:160-168.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13909