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Original Research Article

Cystatin C- an early diagnostic biomarker of diabetic kidney disease

ABSTRACT

Aims: This study was aimed to find out better biomarker between serum Creatinine and serum cystatin C, which will help in 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 to 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. Serum Creatinine and serum cystatin C were estimated. Results were analysed by unpaired t-test & P-value was determined.

Results: Serum cystatin C was found to be increased in microalbuminuria patients as compared with controls and difference was statistically significant (*P = 0.000*). We also found increased levels of serum creatinine which was not significant (*P = 0.11*).

Conclusion: Diabetes has become the most common single cause of end stage renal disease (ESRD) worldwide. The results of this study suggest that serum cystatin C measurement is a useful, practical, non-invasive tool for the evaluation of renal involvement in the course of diabetes, as serum creatinine values are affected by many factors like age, sex, muscle mass, diet and 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 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

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stage renal disease (ESRD) worldwide[5]. About 20-30% of patients with type 1 or type 2 diabetes mellitus develop evidence of nephropathy, but in type 2 diabetes, a considerably smaller fraction of these progresses to ESRD. However, because of much higher prevalence of type 2 diabetes mellitus, 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 (≥ 30mg to 299 mg/day) albumin in urine (stage of microalbuminuria) to stage of macroalbuminuria / clinical albuminuria (≥ 300mg/day) to ESRD. Progress from microalbuminuria to macroalbuminuria usually takes 10-15 years. It remains underdiagnosed in the diabetic population. Type 2 DM may be present for decade before diagnosis and patient may present with complications at the time of diagnosis. So early recognition of diabetic kidney disease by health care professionls is vital for proper management.

Although serum creatinine has been widely used as a marker, but 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 number of investigations are available, many of these are not done in public hospitals, thereby missing diagnosis of diabetic kidney disease at early stage. This could be due to non availability of resources, manpower or funds. This current study is aimed to evaluate the levels of serum Creatinine and serum cystatin C in type 2 diabetic patients.

2. MATERIAL AND METHODS

The study was conducted in LTM Medical College, Sion, Mumbai after the approval from ethics committee. The diagnosed patients of type 2 diabetes mellitus attending medicine OPD at LTM Medical College, Sion, Mumbai were enrolled for the study. On fulfilling the criteria, the study design was explained to the patients with the help of information sheet. Then the written consent was taken.

24 hrs urine sample was tested for proteinuria by using TCA method. Patients having frank proteinuria were excluded. Patients negative for frank proteinuria were further tested for microalbuminuria. In study group, we included 50 patients having microalbuminuria and in control group, 50 patients without microalbuminuria.

Urine albumin by immunoturbidimetric method[7,8], Serum creatinine by modified Jaffe's method[9], Serum cystatin C by immunoturbidimetry[10] were estimated. Patients having frank proteinuria , any known renal disease, taking any medications affecting renal functions, patients with urinary tract infections or hematuria or febrile illness, patients having paraplegia, muscle wasting disease or any neuromuscular disorders were excluded.

Statistical Analysis :

For each parameter to be studied , mean and standard deviation was calculated to estimate the significance . The difference between the groups was measured by Students Unpaired 't' test. P Value less than 0.01 considered as statistically significant.

3. RESULTS AND DISCUSSION

Table shows a non-significantly ($p = 0.11$) 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).

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There was a significant ($p < 0.01$) 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).

Comparison of Serum Creatinine and Serum Cystatin C:

Parameter	Controls(n= 50)	Cases(n= 50)	*P* value
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

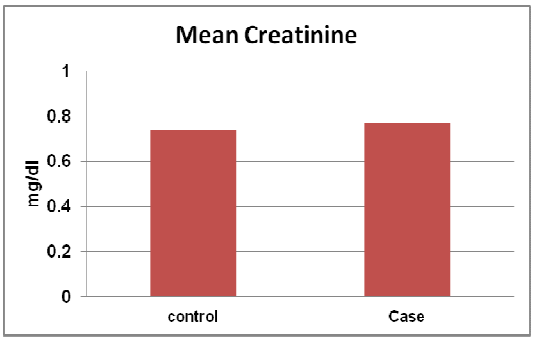

Fig.1. Comparison of Serum creatinine between Control and Study group

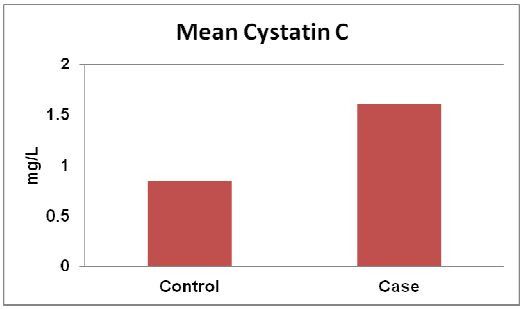

Fig. 2. Comparison of Serum Cystatin C between Control and Study group

DISCUSSION:

Overt diabetic nephropathy classically begins with dipstick-positive proteinuria (urinary albumin excretion > 300 mg/24 h) followed or accompanied by the development of hypertension and/or falling glomerular filtration rate[11,12]. At this stage, renal lesions of

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diabetes, including diffuse mesangial expansion and consequent loss of filtration surface, arteriolar hyalinosis, and global glomerular sclerosis are advanced, and the progression toward endstage renal disease may be slowed but not reversed[13]. Overt nephropathy is preceded by a variable period, which has been called 'incipient nephropathy'[12].

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 but less than the levels that usually give positive dipstick results (30-300 mg/24 hrs). 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/24 h[14-17].

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[18]. Before these stages 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.

Microalbuminuria has been shown by several groups of investigators to indicate a high risk for the later development of overt nephropathy. Studies have shown that urinary albumin excretion in the range agreed by most investigators to be associated with increased nephropathy risk is frequently associated with reduced GFR and more severe diabetic glomerular pathology. Thus, 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 increases in albuminuria (microalbuminuria) precede the development of clinical diabetic nephropathy by several years[19]. The concept of a prognostic role for microalbuminuria has been extended to suggest that normoalbuminuria ensures no reduction of renal function[20]. Based on this, we divided type 2 diabetic patients in 2 groups depending on their diferent 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 S. Creatinine and S. Cystatin C in a small cohort of patients with type 2 diabetes 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[21]. 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[22] , Min Zhang et al[23] , Laura Pucci et al[24] , Jan Kyhse-Andersen et al[25] and Radovan Hojs et al[26] .

The routine classical evaluation of diabetic kidney disease includes appearance of microalbuminuria, decreased creatinine 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[27].

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[28]. Our study

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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 markers of renal involvement[28]. 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 with native kidney disease, and pediatric patients with native kidney disease. They found that serum Cystatin C was superior to serum creatinine[29].

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 and accuracy of using serum cystatin C levels and compared these results to those obtained using traditional renal function indicator such as serum creatinine. Our study found that the diagnostic accuracy of serum cystatin C levels was superior to that of 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 levels has some advantages over serum creatinine, in that serum Cystatin C levels are independent of age, gender, muscle mass,diet, inflammatory status and renal tubular secretion[30]. So serum cystatin C can be considered to be an ideal indicator of GFR.

4. CONCLUSION

In conclusion, the results of this study suggest that serum cystatin C measurement is a useful, practical, non-invasive tool for the evaluation of renal involvement in the course of diabetes. Though urine albumin to creatinine ratio was statistically significant, it has been reported that a decline in the renal function of patients with diabetes is not always accompanied by an increased urine albumin to creatinine ratio, as both albumin and creatinine values are affected by many things 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 end stage renal disease. 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.

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