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Original Research Article

Cystatin C in the early diagnosis of diabetic nephropathy and its correlation with albuminuria

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ABSTRACT

Background: Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia. The metabolic dysregulations associated with DM causes secondary pathophysiological changes in multiple organ systems which result in various complications, responsible for the morbidity and mortality associated with the disease.
Methods: The present study was undertaken in the Department of Medicine in collaboration with the Department of Biochemistry, of SGRDIMSR, Vallah, Sri Amritsar, Punjab, India. The present hospital based study was undertaken with a total number of 100 patients.
Results: The mean Cystatin C values in Group A were 1.73 and mean Cystatin C values in group B were 2.07. The results show that the Cystatin C values were raised even in the patients in whom clinical albuminuria had not yet started.
Conclusions: serum Cystatin C may be considered as an early marker, than microalbuminuria and serum creatinine, the commonly used marker for nephropathy, for declining renal function, in diabetic subjects. Further studies in larger population are needed to confirm this result.

Keywords: Albuminuria, Cystatin C, Diabetes Mellitus, Diabetic nephroapthy

INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia. The metabolic dysregulations associated with DM causes secondary pathophysiological changes in multiple organ systems which result in various complications, responsible for the morbidity and mortality associated with the disease.[1] The estimated number of adults with diabetes in 2007 was 246 million. 80% of this population lives in developing countries, the largest numbers in the Indian subcontinent and in China. Approximately 85-95% of all cases of diabetes are type 2 diabetes and the worldwide explosion of this disorder is a major health care burden. It is estimated that nearly 380 million adults worldwide will have diabetes by 2025. India has 41 million diabetics IN 2012 and this number is expected to increase to 70 million by 2025.[2] The recent steep rise in the prevalence of type 2 diabetes worldwide, which is extremely pronounced in Asian countries and is particularly dramatic in India, has given India the dubious distinction of the "diabetes capital of the world". In parallel with the increase in diabetes, an increase in the prevalence of one of the microvascular complication - diabetic nephropathy has also been noted which has become the single most common cause of end-stage kidney disease.[3] Even when diabetes is controlled, the disease can lead to chronic kidney disease (CKD) and kidney failure.[4] Unfortunately, it has also been reported that CKD is significantly under diagnosed and

undertreated.[5] The disease is clinically silent until its late stages, at which point patients may suffer significant irreversible damage or mortality from their renal impairment in the absence of screening and intervention. Earlier detection will not only help in the clinical management of patients but also spur new research into therapies for kidney disease.[6]

In an effort to improve early diagnosis, the National Kidney Foundation has issued standardized clinical practice guidelines according to the Kidney Disease Quality Initiative. In these guidelines and recommendations the primary measure of renal function is the glomerular filtration rate (GFR).[7] GFR is best measured by injecting compounds such as inulin, radioisotopes such as 51 chromium-EDTA, 125 I-iothalamate, 99 m Tc- DTPA or radio contrast agents such as iohexol, but these techniques are complicated, costly, time-consuming and have potential side-effects.[8] Creatinine is the most widely used biomarker of kidney function. It is inaccurate at detecting mild renal impairment, and is also non-specific as its levels can vary with muscle mass and protein intake of the patients.[9] The presence of microalbuminuria is known to precede an elevation in serum creatinine by several years. The presence of microalbuminuria has proven to be a powerful screening tool and biomarker to detect diabetic patients at risk for diabetic nephropathy. It is now a standard of care to screen annually for the presence of microalbuminuria in all patients with DM.[6] Moreover, impaired renal function may be present even in the patients with normal urinary albumin excretion rate.[10] This suggests a need to screen patients many years before the onset of microalbuminuria. Urine proteomic profiling has enabled researchers to detect potential biomarkers in patients with type 2 diabetes up to 10 years before the onset of microalbuminuria.[6] As new biomarkers are discovered, clinicians will be able to screen for diabetic nephropathy many years earlier than they now can.

The limitations of above discussed biomarkers have led to an extensive search for a more sensitive laboratory marker of impaired renal function. The ideal GFR marker should be an endogenous molecule which, being produced at a constant rate, is cleared solely by the kidneys via free glomerular filtration, with being neither secreted by tubular cells, nor reabsorbed into peritubular circulation.[11] A number of proteins with molecular weights of less than 30 kD possess these qualities and include α2-microglobulin, retinol-binding protein (RBP), α1-microglobulin, β-trace protein and Cystatin-C.

These proteins are filtered at the glomerulus, then reabsorbed (and metabolized) in the proximal tubule or excreted into the urine, and thus they are entirely eliminated from the circulation. However, apart from Cystatin-C, all the other proteins have been shown to have serum concentration that are influenced by non-renal factors. 12A number of studies have explored the clinical utility of plasma Cystatin-C as a diagnostic marker of GFR.[13-14] The human cystatin family presently comprises 11 identified proteins. Two of these, Cystatin A and B, form the family 1 Cystatin and are mainly, or exclusively, intracellular proteins, while Cystatin C, D, E, F, S, SA and SN are mainly extracellular and/or transcellular proteins and constitute the family 2 Cystatins. The family 3 Cystatins, high and low molecular weight kininogen, contain three Cystatin domains and are mainly intravascular proteins, which in addition to being inhibitors of cysteine proteases also are involved in the coagulation process and in the production of vasoactive peptides.[15] Cystatin-C, a 13 kD protein is produced by a housekeeping gene in all nucleated cells. This cysteine protease inhibitor is freely filtered by the glomerulus without stearic restrictions, and does not appear to be secreted by the renal tubules.[16] Its low molecuar weight and positive charge at physiological pH facilitate its glomerular filtration. Subsequently, it is reabsorbed and almost completely catabolized in the proximal renal tubule. Therefore, because of its constant rate of production, its serum concentration is determined by glomerular filtration.[17] If kidney function and GFR decline, the blood levels of Cystatin-C rise. Serum levels of Cystatin-C are a more precise test of kidney function (as represented by the GFR) than serum creatinine levels. 7It has been suggested to be closer to the "Ideal" endogenous marker.[17]

Advantages of Cystatin-C as a biomarker for detection of renal functions over other available markers:

- Serum Cystatin-C does not depend on muscle mass, diet, or gender.[12] Like plasma Cystatin-C, urinary Cystatin-C is unaffected by age or muscle mass[16]
- There are no known extra renal routes of elimination and clearance from circulation is only by glomerular filtration
- 7Cystatin-C is sensitive to changes in the so-called creatinine blind GFR range (40-70ml/min/1.73m^2)
- Cystatin-C concentration is not influenced by infections, liver diseases, or inflammatory diseases.18It can also be used to detect and monitor kidney disease in patients with hepatic disease.[6]

METHODS

The present study was undertaken in the Department of Medicine in collaboration with the Department of Biochemistry, of SGRDIMSR, Vallah, Sri Amritsar, Punjab, India.

The present hospital based study was undertaken with a total number of 100 patients attending Department of Medicine, SGRDIMSR, Vallah, Sri Amritsar, Punjab, India, diagnosed as type 2 diabetes mellitus (DM) after approval from Institutional Thesis and Ethical Committee. Relevant history bio-data and consent of the patient was recorded as per proforma attached.

The patients will be divided into 2 equal groups.

Group A

Type 2 diabetes with normoalbuminuria.

Group B

Type 2 diabetes with microalbuminuria.

Inclusion criteria

- Known cases of type 2 DM for more than 5 years.
- Age of 30 years and above.

Exclusion criteria

- Thyroid dysfunction
- Patients on glucocorticoid therapy
- Hypertension
- Past history of renal impairment.

Serum Cystatin-C levels will be estimated by enzyme linked immunosorbent assay (ELISA) as described by Pergande M.

Reference intervals for serum Cystatin-C

- Adults (male or female; 20-50 years): 0.70 - 1.21 mg/L
- Adults (male or female; above 50 years): 0.84 - 1.55 mg/L

Calculation of albumin/creatinine ratio

- Urine sample will be collected in a sterile container and will be analysed for following constituents:
- *Albumin:* Estimation will be done by pyrogallol red method.
- *Creatinine:* Estimation will be done by Jaffe's alkaline picrate method.
- Normoalbuminuric/microazbuminuric patients will be detected by calculating the urine albumin creatinine ratio (ACR) as following
- ACR = Concentration of Albumin/Concentration of creatinine (µg albumin/g creatinine)
- ACR < 30µg/g - normoalbuminuria
- = 30-300µg/g - microalbuminuria

RESULTS

The mean Cystatin C values in Group A were 1.73 and mean Cystatin C values in Group B were 2.07. The results show that the Cystatin C values were raised even in the patients in whom clinical albuminuria had not yet started. Cystatin C values are seen to be raised in people where clinical albuminuria has not even started yet and hence acts a earlier marker than microalbuminuria to determine nephropathy. Also Cystatin C acted like an alternate marker of nephropathy to albuminuria in patients who had decreased GFR.

Table 1: Mean cystatin C values in both groups.

	Group A	Group B
Mean	1.73±1.14	2.07±1.06
t-value	-1.538	
p-value	0.127	

DISCUSSION

The results of the current study demonstrated that cystatin-C values were raised in the patients with normoalbuminuria and mean value was 1.73 mg/L vs 2.07 mg/L in microalbuminuric patients.

This suggests that cystatin -C acts as a marker even before microalbuminuria begins.[19] Yun Kyung Jeon et al, studied 335 T2DM patients. These patients were divided into normoalbuminuric, microalbuminuric and macroalbuminuric patients. In normoalbuminuric patients, cystatin C levels of serum were significantly increased in patients with GFR < 60 mL/min/1.73m2. This suggests that Cystatin C levels of serum are related to subclinical tubular impairment and can be an earlier subclinical marker of renal involvement before onset of albuminuria. Takir M et al. studied 78 T2DM patients and they divided into 4 groups depending on their urine albumin excretion and eGFR.[20] The values of Cystatin C were significantly increased in the normoalbuminuria group which shows its value as an early marker of diabetic nephropathy. The serum cystatin C level of microalbuminuric patients was found to negatively correlate with eGFR (r=-0.892, p=0.001). This shows that serum cystatin C is an early marker of nephropathy.[21] Tan et al concluded cystatin C is a more reliable measure of GFR than creatinine clearance, is more highly correlated with iohexol clearance than plasma creatinine, and is worthy of further investigation as a clinical measure of GFR in type 1 diabetes.[22] Bruce et al concluded that serial measures of serum cystatin C accurately detect trends in renal function in patients with normal or elevated GFR and provide means for studying early renal function decline in diabetes.[23] Laura et al concluded that use of cystatin C to measure renal function will optimize early detection, prevention and treatment strategies for diabetic nephropathy.

CONCLUSION

The study was conducted in 100 diabetic subjects to study the correlation between serum creatinine, microalbuminuria and serum Cystatin C with diabetic nephropathy. A majority of subjects with proteinuria were found to be associated with comorbid conditions, poorer glycemic control and more diabetic complications. Serum creatinine showed a significant correlation with the albuminuria and the reduced GFR groups. Serum cystatin C also showed a significant correlation with albuminuria and reduced GFR groups. It was found that majority of subjects with lesser duration of diabetes had normal creatinine, normalbuminuria, normal to reduced

GFR but elevated Cystatin C levels in few of them. Results of this study show that serum Cystatin C may be considered as an early marker, than microalbuminuria and serum creatinine, the commonly used marker for nephropathy, for declining renal function, in diabetic subjects. Further studies in larger population are needed to confirm this result.

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