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STUDY ON ASEESSMENT OF CYSTATIN C LEVELS AS EARLY MARKER FOR RENAL IMPAIRMENT IN TYPE 2 DIABETIC SUBJECTS

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ABSTRACT

Background: Diabetic Nephropathy is the leading cause of end-stage-renal disease (ESRD) worldwide and is predominantly due to increasing prevalence of type 2 Diabetes Mellitus. It is clinically characterized by decline in glomerular filtration rate (GFR) and increasing rates of urinary albumin excretion starting from normoalbuminuria which progresses to microalbuminuria, macroalbuminuria and eventually to ESRD.

Objectives of the study: To estimate and compare serum cystatin levels between controls and type 2 diabetic subjects and to study the diagnostic accuracy of cystatin c in assessing the renal impairment.

Materials and Methods: A total number of 90 patients were studied comprising of 30 controls and 60 proven cases of type 2 diabetic patients. Serum concentrations of Cystatin C, Creatinine, Fasting Serum Glucose and Urine Albumin Creatinine Ratio were measured. The statistical analysis was carried out using by Student t-test and Diagnostic Validity Test.

Results: The levels of serum cystatin C, creatinine and ACR were found to be significantly increased in diabetic patients compared to controls ($p < 0.001$). We also found that the diagnostic accuracy of serum cystatin C was found to be better as compared to serum creatinine in assessing early renal impairment in diabetic patients.

Conclusion: Our Study suggests that serum cystatin C allows early diagnosis of decline in GFR and is superior to creatinine in assessing renal glomerular function. So, it can be used for early screening and diagnosis of diabetic nephropathy.

KEYWORDS : Diabetic Nephropathy, eGFR, Cystatin C, Creatinine and Urine Albumin Creatinine Ratio

INTRODUCTION

India has the largest number of diabetic patients in the world, estimated to be approximately 40.9 million in the year 2007 and expected to increase to approximately 69.9 million by year 2025 [1]. Diabetic Nephropathy is the single most leading cause of chronic renal failure. Diabetic nephropathy is an acquired sclerotic injury associated with thickening of the glomerular basement membrane secondary to long standing effects of hyperglycemia, advanced glycation end products and reactive oxygen species [2]. Diabetic nephropathy is clinically characterized by increasing rates of urinary albumin excretion starting from normoalbuminuria, which progress to microalbuminuria, macroalbuminuria and eventually to end stage renal disease [1]. So, it is important to assess renal function as accurately as possible, because renal disease has different clinical presentations and patients are often asymptomatic [3].

GFR is a useful index to assess kidney function [3]. GFR can be measured directly by clearance studies of exogenous markers such as Iohexol, Iothalamate and Cr^{51}-EDTA but these are costly, time consuming, labor-intensive and require administration of substances that make them incompatible with routine monitoring [4]. Endogenous marker plasma creatinine and urea concentration despite their limitations are commonly used because they are cheap and easily available [5]. Urea is freely filtered by the glomerulus and not secreted by the tubules [6]. However, a large portion (40-70%) is passively reabsorbed from renal tubules so underestimate GFR and its concentration in plasma may vary depending on diet, hepatic function and state of numerous diseases [5]. Serum creatinine is an insensitive indicator of diminished GFR because its concentration is affected by meat intake, gender, muscle mass, malnutrition and ageing [6]. Serum creatinine is freely filtered by glomerulus, not reabsorbed by the proximal tubules but is secreted in small amounts leading to over estimation of GFR [5].

Recently Cystatin-C as a surrogate endogenous marker for estimating early decline in GFR in diabetes [7]. Cystatin-C is a low molecular weight of 13 kDa protein belonging to cystatin super family consists of 120 amino acids and its gene located on chromosome 20 [7]. Its concentration is not significantly affected by age, gender, race, muscle mass, diet, infection, liver function, malignancies, myopathies and body fat content and has a desirable traits as a marker because filtered solely by the glomerulus, not secreted by renal tubules, completely reabsorbed by the tubules and then completely catabolized and generated at a constant rate by all cells in body [7]. The present study will be undertaken to evaluate the levels of cystatin-C as a marker of early renal impairment in type-2 diabetic patients in turn helping in the early intervention and management of diabetic nephropathy cases.

OBJECTIVES OF THE STUDY

1. To estimate and compare serum cystatin levels between controls and type 2 diabetic subjects
2. To study the diagnostic accuracy of cystatin c in assessing the renal impairment.

MATERIALS AND METHODS

Source of data: The present study was carried out for a period of one year from 2012 -2013. The patients will be selected from Chigateri General Hospital and Bapuji Hospital, Davangere (both hospitals are attached to the teaching institute J.J.M. Medical College, Davangere). The study was carried out in type-2 diabetic patients and age and sex matched healthy controls. Both cases and controls will be interviewed to obtain relevant data.

Inclusion Criteria:

Cases: 60 proven cases of type-2 diabetic patients in age group of 30 - 80 years.

Controls: 30 cases of age and sex matched healthy controls will be compared.

All patients suffering from type-2 diabetes diagnosed and confirmed by physician with FBS and PPBS according to American Diabetes Association criteria (FBS ≥126 mg/dl & 2 hour PPBS ≥ 200 mg/dl).

Exclusion criteria: Patients with thyroid disorders, under thyroid medications, under steroid therapy, uncontrolled hypertensive patients and cardiovascular disease patients.

Method of data collection: After obtaining informed consent, about 5ml of fasting venous blood samples will be drawn under aseptic precautions in to a sterile bulb from selected subjects. Serum will be separated by centrifugation. Parameters were estimated by the following methods:

Serum cystatin-C by Immunoturbidimetry, Serum creatinine by Jaffe's Method, Urinary microalbumin by Immunoturbidometry, Albumin by Bromocresol Green method, Serum Glucose by Hexokinase method.

Statistical analysis: Results will be subjected for appropriate statistical analysis.

1) Student t test was used for the comparison between cases and controls.
2) Diagnostic validity test such as specificity, sensitivity, positive predictive value, negative predictive value will be used to assess the utility of these parameters as biomarkers in diabetic nephropathy and to differentiate type-2 diabetic cases from healthy controls.

RESULTS

The results obtained in this present study were from total number of 90 subjects. These include healthy controls 30 and Diabetic patients 60. Out of 30 Healthy Controls 22 were males and 8 were females and out of 60 Diabetic patients 36 were males and 24 were females. The mean in controls was 56.8 9.6 and in type 2 diabetic subjects was 56 12.6.

Table 1: Shows Comparisions Of Serum Cystatin C, Creatinine, Urine Albumin: Creatinine Ratio And Fasting Blood Sugar Between Controls And Type 2 Diabetic Subjects

	CONTROLS [n = 30]	CASES [n = 60]	p Value
Cystatin C (mg/L)	0.72 0.14	2.16 1.25	<0.001
Creatinine (mg/dL)	0.81 0.13	1.80 1.30	<0.001
FBS (mg/dL)	94.7 7.1	278.4 88.7	<0.001
UCAR (mg/g)	18.25 7.11	618.9 692.6	<0.001

It is evident from the table 2 that the levels of serum cystatin c, creatinine, urine albumin: creatinine ratio and fasting blood sugar are elevated in type 2 diabetic subjects compared to controls. This increased levels are statistically highly significant.

Table 2: Shows Comparision Of Diagnostic Validity Between Cystatin C And Creatinine

(Diagnostic validity of cystatin C in predication of renal impairment in diabetic patients with cut off level of 0.90 mg/L)

CYSTATIN C (mg/L)	CASES	CONTROLS	TOTAL
<0.90	1	28	29
>0.90	59	2	61
TOTAL	60	30	90

(Diagnostic validity of creatinine in predication of renal impairment in diabetic patients with cut off level of 1.02 mg/L)

CREATININE (mg/dL)	CASES	CONTROLS	TOTAL
>1.2	43	2	45
<1.2	17	28	45
TOTAL	60	30	90

VARIABLE	CYSTATIN C	CREATININE
X^2	76.95	33.80
p Value	<0.001	<0.001
Sensitivity	98%	72%
Specificity	93%	93%
Positive Predictive Value	97%	96%
Negative Predictive Value	97%	62%
Diagnostic Efficiency	97%	79%

It is evident from the above tables that the serum cystatin C has more sensitivity, specificity, PPV, NPV and Diagnostic Efficiency as compared to serum creatinine for detecting renal impairment in type 2 diabetic subjects.

DISCUSSION

Diabetes Mellitus is the commonest endocrine metabolic disorder, expected to increase to approximately 69.9 million by year 2025. Uncontrolled glycemic control leads to various acute and chronic complications in diabetic subjects. Diabetic nephropathy is an important microvascular complication seen in type 2 diabetic subjected exposed to chronic hyperglycemia. Early screening and diagnosis of diabetic nephropathy is very important in these subjects. Till date, the traditional markers of renal disease like creatinine and urea were estimated in the serum sample, since these tests are cheap and easily available. But still these traditional markers are used widely, but they have several limitations like Urea is freely filtered by the glomerulus and not secreted by the tubules. However, a large portion (40-70%) is passively reabsorbed from renal tubules so underestimate GFR and its concentration in plasma may vary depending on diet, hepatic function and state of numerous diseases. Serum creatinine is an insensitive indicator of diminished GFR because its concentration is affected by meat intake, gender, muscle mass, malnutrition and ageing and one of the most important concern with serum creatinine is that, it will not be elevated until 50% of the kidneys are damaged. So, we are need of a marker which can the renal impairment earliest hence the appropriate therapeutic approach can be installed hence, progression to nephropathy can be prevented [5,6].

In this study, we found elevated fasting blood sugar, creatinine, cystatin C and urinary albumin creatinine ratio in diabetic subjects as compared to controls. The elevation was statistically significant. When we employed diagnostic validity test between elevation in serum creatinine and serum cystatin, serum cystatin levels were elevated in 98.33% of the cases compared to creatinine elevation which was about 71.6% of the cases almost underestimating about 26.7% of the cases. Cystatin C had increased sensitivity (98%) and negative predictive (97%) value compared to creatinine which was about 72% and 62% respectively. Cystatin C is a 13 KDa protein produced virtually by almost all nucleated cells, its production is constant and not affected by inflammatory process, gender, age and muscle mass. It is freely filtered, completely reabsorbed and degraded by proximal tubular cells. Therefore, serum concentration of cystatin C is almost exclusively determined by the GFR, making Cystatin C as excellent indicator of GFR. It is more accurate than serum creatinine. This finding was similar to the studies conducted in the past [8,9,10,11,1213].

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