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Study on Cystatin C An early biomarker for Nephropathy in Type II Diabetic subjects

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Abstract:

Type 2 diabetes has become the most common metabolic disorder in India and is a growing problem with over 40 million diabetic subjects. The "Asian Indian phenotype" is associated with increased insulin resistance, greater abdominal adiposity despite lower body mass index, lower adiponectin and higher high sensitivity C - reactive protein levels makes Asians more prone to diabetes. Among Indians, the onset of type 2 diabetes occurs at a younger age and hence, they are vulnerable to all the complications of diabetes due to longer duration of the disease. An overnight fasting blood sample was collected from both cases and controls and the samples were centrifuged and separated for the estimations. Estimations of fasting blood glucose, blood urea and serum creatine were performed. Estimation of serum cystatin C was done by immunoturbidimetric method. The care of patients with diabetes and end stage renal disease contributes significantly to health care costs. In the past couple of decades, there have been notable advances in our knowledge regarding the early stages of diabetic kidney disease, including the advent of interventions that can significantly slow or even reverse the progression of disease. The limitations of currently available parameters in early detection of renal dysfunction have prompted a search for newer, more reliable markers of renal function. This study attempts to determine the utility of serum Cystatin C in predicting early decline in renal function so that appropriate and timely interventions can be instituted to delay or arrest the progression of diabetic nephropathy.

Key words: Type 2 DM, cyctatin C, diabetic nephropathy, albuminuria

Introduction:

Type 2 diabetes has become the most common metabolic disorder in India and is a growing problem with over 40 million diabetic subjects. The "Asian Indian phenotype" is associated with increased insulin resistance, greater abdominal adiposity despite lower body mass index, lower adiponectin and higher high sensitivity C - reactive protein levels and makes Asians more prone to diabetes. Among Indians, the onset of type 2 diabetes occurs at a younger age and hence, they are vulnerable to all the complications of diabetes due to longer duration of the disease.

Diabetic nephropathy is one of the leading cause of chronic renal failure in India contributing to over 30% of cases.[1] In the last 50 years, blood urea and

blood urea and serum creatinine estimation have become the most commonly used serum markers of renal function. Urea concentration in the blood can vary with diet, hepatic function and numerous disease states. Furthermore, rate of appearance of serum creatinine in the blood stream is related to muscle mass and its blood concentrations are affected by diet, age, race and gender. As plasma concentrations increase, tubular secretion of serum creatinine increases, leading to an overestimation of GFR. Serum creatinine is also insensitive for detecting small decreases in GFR because of the nonlinear relationship between plasma concentration and GFR.[2]

Microalbuminuria can detect early diabetic nephropathy. However, short-term hyperglycemia, exercise, urinary tract infections, marked hypertension, heart failure and acute febrile illness can cause transient elevations. There is also marked day-to-day variability in albumin excretion.[3]

The care of patients with diabetes and end stage renal disease contributes significantly to health care costs. In the past couple of decades, there have been notable advances in our knowledge regarding the early stages of diabetic kidney disease, including the advent of interventions that can significantly slow or even reverse the progression of disease. The limitations of currently available parameters in detecting renal dysfunction early have prompted a search for newer, more reliable markers of renal function.

This study attempts to determine the utility of serum Cystatin C in predicting early decline in renal function so that appropriate and timely interventions can be instituted to delay or arrest the progression of diabetic nephropathy.

Material and Methods:

The study will comprise cases of Type 2 Diabetes Mellitus visiting the inpatient and outpatient of G. R. Medical College Gwalior (M.P.). A part of the analysis of blood has been carried out at Laboratory Medicine, Department of Biochemistry, Medanta The Medicity Hospital, Gurgaon. The study was carried out in 300 subjects out of which 100 subjects were treated as controls groups and 200 subjects were of Type 2 Diabetes Mellitus suffering from nephropathy. Age and sex matched healthy volunteers will serve as control.

Inclusion criteria: Cases of Type 2 Diabetes Mellitus suffering from nephropathy, hypertension, obesity etc.

Exclusion criteria: Patients on glucocorticoids, nephrotoxic drugs, endocrine disorders, rheumatoid disease, malignancy, fever, dehydration.

Before starting analysis the written consent from all subjects were taken. The study was approved by institutional ethical committee and was carried out by keeping all norms in mind.

The clinical manifestations of disease, personal history of patients were recorded in study proforma.

Collection of Blood Sample:

An overnight fasting blood sample was collected from both cases and controls and the samples were centrifuged and separated for the estimations. Estimations of fasting blood glucose, HbA1C (Glycosylated Hb), blood urea and serum creatine were performed using the serum. Estimation of serum cystatin C was done by immunoturbidimetric method.

Statistical analysis:

Data were analyzed by SPSS student t-test and one way ANOVA. P-value <0.05 was considered statistically significant.

Results and Discussion:

Table 1 shows the clinical characteristics of diabetic nephropathy subject groups and control groups, there is a significant difference (P<0.05) in WHR in diabetic patients with normal-albuminuria as compared to other study group. There was a positive correlation between serum cystatin C and serum creatinine (r= 017) among cases in this study. This is in conformity with a study done by Buysscheart M et al. who found a linear relationship

between serum cystatin C and serum creatinine (r=0.92).[4]

Table No. 1 (a), (b), (c) Characteristics of control, group I and group II diabetic nephropathy subjects. (Physiological parameters)

1(a)

Healthy Control (n=100)					
Parameters	**Min**	**Max**	**Mean**	**±SD**	**±SE**
AGE	29	73	50.2	10.3	1.03
BMI	18.9	24.9	22.04	1.4	0.14
Systolic BP	100	120	115.7	4.6	0.46
DiastolicBP	70	80	76.6	3.92	0.39
GFR	70	142	112.11	13.37	1.33
HB	8.2	18.7	13.79	1.63	0.16
WBC	4.67	17	7.3	7.57	0.75
RBC	2.72	6.76	4.91	0.58	0.058

1(b)

Micro-albuminuria Group I (n=100)					
Parameters	**Min**	**Max**	**Mean**	**±SD**	**±SE**
AGE	35	79	56.16	10.47	1.04
BMI	18.7	35.3	25.7	4.15	0.41
Systolic BP	120	160	135.7	11.13	1.11
DiastolicBP	50	90	70.72	12.09	1.20
GFR	50.5	101.1	77.37	14.22	1.42
HB	6.3	17.6	13.27	2.09	0.20
WBC	0.58	33.25	8.67	4.72	0.47
RBC	2.13	6.61	4.70	0.77	0.07

1(c)

Macro-albuminuria Group II (n=100)					
Parameters	**Min**	**Max**	**Mean**	**±SD**	**±SE**
AGE	42	82	60.39	9.13	0.91
BMI	18	40.2	26.45	4.75	0.47
Systolic BP	120	180	146.3	14.5	1.45
DiastolicBP	50	90	79.83	10.44	1.04
GFR	34	80	51.03	11.2	1.12
HB	7.5	18	12.89	2.5	0.25
WBC	4.43	30.2	8.62	4.88	0.81
RBC	2.92	6.55	4.88	0.81	0.08

*Significant at (p<0.001)

Table No. 2 (a), (b), (c) Showing the comparative changes of biochemical and renal parameters in control group and diabetic nephropathy group I and group II subjects.

2(a)

Control (n=100)					
Parameters	**Min**	**Max**	**Mean**	**±SD**	**±SE**
FBS	73	124	94.93	9.47	0.94
HbA1C	4	6.7	5.38	0.69	0.06
UREA	19	38	25.77	5.47	0.05
CREA	0.5	1.0	0.81	0.14	0.001
Cys-C	0.29	0.98	0.64	0.18	0.01

2(b)

Micro-albuminuria Group I (n=100)					
Parameters	**Min**	**Max**	**Mean**	**±SD**	**±SE**
FBS	96	265	170.05	40.31	4.03
HbA1C	6.4	13.8	8.66	1.59	0.15
UREA	16	46	27.32	5.87	0.58
CREA	0.5	1.5	1.07	0.23	0.02
Cys-C	0.95	2.91	1.63	0.46	0.04

2(c)

Macro-albuminuria Group II (n=100)					
Parameters	**Min**	**Max**	**Mean**	**±SD**	**±SE**
FBS	120	303	184.89	47.97	4.79
HbA1C	6	13.75	9.21	1.59	0.15
UREA	18	77	40.07	12.63	1.26
CREA	0.8	3.8	1.62	0.67	0.06
Cys-C	0.98	3.5	2.06	0.59	0.05

* Significant at (p<0.001)

Several low molecular weight proteins have been evaluated as endogenous markers of GFR with Cystatin C commanding the most attention. Cystatin

C is a 132-amino acid, 13-kDa cysteine protease inhibitor produced by all nucleated cells, whose function is thought to be modulation of the intracellular catabolism of proteins. It is formed at a constant rate and is freely filtered by the renal glomeruli, fulfilling an important criterion for any endogenous marker of GFR.[5] Plasma Cystatin C values are reported to be unaffected by age, body weight, diet, medications or pathologies such as inflammation.[6] Dharnidharka VR et al performed a meta-analysis of 46 previous studies involving 4496 subjects, published in the American Journal Of Kidney Disease 2002, to compare the accuracy of Cys C and Creatinine in relation to a reference standard of GFR. The study established the overall coefficient of correlation r was significantly greater for 1/cystatin c (mean r = 0.816) in comparison to 1/creatinine (mean r = 0.742) at p value <0.001.[7] A Belgium- based study by Willems D et al that compared renal markers to Cr-EDTA clearance in 67 diabetic patients with normal creatinine showed that Cystatin C was a more sensitive parameter than creatinine for the detection of an incipient nephropathy in diabetes.[8] Surendar J et al studied 209 subjects at the Madras Diabetes Research Foundation in the year 2008. The subjects were divided into 5 groups depending on glucose tolerance, presence or absence of nephropathy and retinopathy. The study concluded that Cystatin C levels increase and Cys-GFR levels decrease with increasing severity of glucose intolerance.[9] Several similar studies in the past decade have established the diagnostic accuracy of serum Cystatin C over serum creatinine for stage 1 and 2 chronic kidney disease.[10] However, there are few studies that have been performed in the Indian subcontinent that support currently available data. Mussap M(2002), Pucci L(2007), Rigalleau V(2008), et al wrote in a study that Cystatin C is produced at a constant rate by nucleated cells and released into bloodstream with a half-life of 2 hrs. Its concentration is almost totally dependent on GFR. Other studies have demonstrated that serum cystatin C is an early renal marker in diabetic patients [11,12,13] but not all studies have done so. Oddoze C (2001) et al[14] Comper WD et al[15] (2003) determined Cystatin C, a cysteine protease inhibitor, is freely filtered by the renal glomeruli, metabolized by the proximal tubule and identified as a promising marker of renal failure. Diabetes has become the most common single cause of end stage renal disease (ESRD). In the U.S., diabetic nephropathy accounts for about 40% of new cases of ESRD. Assessment and follow up of early renal dysfunction is important in diabetic nephropathy. Cystatin C concentration has been proposed as an endogenous marker of GFR superior to creatinine.[16] The routine classical evaluation of diabetic nephropathy includes appearance of microalbuminuria, decreased creatinine clearance and increased serum creatinine.[17] Others studies suggested that several tubular markers increase more in diabetic patients than in healthy controls, and this correlated with the severity of albuninuria.[18] Serum cystatin C is the most valid marker to estimate the GFR, rather than serum creatinine and to predict progression of renal dysfunction.[19]

Conclusion:

These findings suggest that cystatin C measurement in urine and serum is a useful, practical, non-invasive tool for the evaluation of renal involvement in the course of diabetes, especially in normoalbuninuric patients. 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 nephropathy. The present study showed that cystatin C was a good marker of impaired renal function and its more sensitive marker in most study groups when compared with creatinine. Cystatin C had good correlation with creatinine.

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