ORIGINAL ARTICLE

Cystatin C versus Creatinine as Early Stage Diabetic Nephropathy Marker

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ABSTRACT

Objective: To compare the levels of serum cystatin and creatinine as the markers of early stage of diabetic nephropathy.

Study Design: Case Control study.

Place and Duration of Study: The study was conducted at Railway Hospital, Rawalpindi for a period of one year from March 15th, 2016 to March 16th, 2017.

Materials and Methods: A total of 77 diabetics and 77 healthy controls were selected. These included adults above 40 years of age. The levels of Serum cystatin C and creatinine were measured using IMAGIN Specific Protein Analyzer. Both tests were done by applying standardized laboratory protocols. The study outcome was determined in terms of detection of diabetic nephropathy which was finalized on the basis of albuminuria status derived as three categories; normoalbuminuric, microalbuminuria and macroalbuminuria.

Results: Male cases were more than females (58.4% vs 41.6%), and were equally distributed in patients and control groups. The levels of Cystatin C (4.7 $\pm$ 3.9 mg/l), and creatinine (1.0 $\pm$ 0.13 mg/dl) were found significantly high in the macroalbuminuria group as compared to controls and normoalbuminuric group (p-value <0.001). Serum cystatin C was significantly raised in microalbuminurics as compared to serum creatinine proving its worth for detecting early stage diabetic nephropathy (p-value <0.001).

Conclusion: Serum cystatin C is a better predictive marker of diabetic nephropathy than serum creatinine.

Key Words: *Diabetic Nephropathy, Serum Cystatin C, Serum Creatinine.*

Introduction

Type 2 diabetes is on a continuous rise worldwide owing to a steady increase in obese and aging population. The global prevalence of diabetes was estimated at 171 million (2.8%) in the year 2000 which is expected to reach 366 million 4.4% figure by the year 2030.[1]

The current prevalence of diabetes in Pakistan is around 10.0%.[2] As per WHO estimates, Pakistan is ranked 7th largest country suffering from diabetes mellitus and it is expected that by 2030 this rank will climb the ladder to 4th position which is an alarming statistics and situation.[3]

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Diabetic nephropathy is one of the most common complications of diabetes. Diabetic nephropathy by definition is macroalbuminuria (albumin excretion rate $\geq$ 300 mg /24 hours) and deteriorating renal function is a known fact in diabetics. Previous reports confirm that approximately one third to one half of diabetic patients develop renal complications.[4]

Albuminuria is a significant prognostic factor for risk stratification of diabetic nephropathy and monitoring of its progression. There was a belief that microalbuminuria is predictive of future overt diabetic nephropathy in 80% cases, however, on the contrary, it has been proposed that around 30% of microalbuminuria cases progress to overt nephropathy after 10 years follow-up.[4,5]

Presently, the phenomena of normoalbuminuric diabetic nephropathy is well established and portrays that diabetic patients may present with a decreased GFR without progression from normal to microalbuminuria.[4] Gold standard method for determining GFR in research settings are inulin and Cr –EDTA plasma clearance. These techniques are time consuming, laborious and requiring expertise making them unfit for clinical practice. Hence the

used index for GFR is serum creatinine (mg/dl). Moreover, its sensitivity is poor in early renal damage and by the time its levels are detectable, significant decrease in GFR has already occurred.[5]

Putting these facts together, there is a ground for identification of alternative biomarkers to predict diabetic nephropathy early so that timely management and maintenance can be exercised. Cystatin C a 13.3k Da plasma protein is relatively new marker in the prediction of renal impairment and it correlates positively with other renal tests like GFR. Serum creatinine also a proven marker of nephropathy is relatively weaker test and easily changes by different maneuvers and circumstances like a person's muscle mass.[5] Cystatin C has been found constant and unaffected and an alternative with high sensitivity for diabetic nephropathy using a cut off of >60 ml of GFR.[5,6]

The focus of research by endocrinologists and other investigators is to find out new and better biomarkers for the diagnosis of early diabetic nephropathy. The aim of the study was to compare cystatin C and creatinine in the screening of diabetic patients on risk of early stage diabetic nephropathy.

Materials and Methods

A case control study was conducted at the Railway Hospital, Rawalpindi from March 15th, 2016 to March 16th, 2017 for one year duration. A measured study sample of 77 diagnosed cases of diabetes were enrolled along with seventy seven normal controls. Convenient sampling technique was utilized. The study was conducted after obtaining permission from ethical review committee. A written informed consent was taken from all the patients. Demographic data was collected via questionnaires. Seventy seven diabetes cases and seventy seven non-diabetics of both genders and adults age (above 18 years) were included in the study.

For study purpose, Albuminuria was divided into three standard operational groups; i) Normoalbuminuria with ACR< 30mg/day, ii) Microalbuminuria with ACR 30 to < 300mg/day and iii) Macroalbuminuria with ACR $\geq$ 300mg/day.

Blood was drawn from peripheral veins, transferred to EDTA tube, gently mixed and made to stand upright. The blood samples were centrifuged at 2200 RPM for 10 minutes. The separated serum was stored at -20°C till completion of sample collection. The urine samples were collected in the jars provided to the patients and centrifuged at 1000 RPM for 10 mins, these were also stored at -20°C till analysis.

The estimation of cystatin C levels (mg/l) was carried out on IMAGIN Specific Protein Analyzer for quantitative determination of human cystatin C in serum. Similarly, the estimation of urinary albumin levels were carried out on IMAGIN Specific Protein Analyzer for quantitative determination of human Microalbumin[MALB] in urine by immunoturbidimetry.[4,5]

Data was analyzed using SPSS 20.0 version. First, descriptive statistics was applied to measure frequency and percentages for categorical variables like gender, and mean and standard deviations for continuous variables. Secondly, using student's t-test the means and standard deviation levels of serum cystatin C, serum creatinine and clinical measurements of blood pressure were compared among patients and controls. Categories of albuminuria were created as per operational definitions. The mean levels of serum cystatin C and creatinine were compared among these categories using T-test. For further analysis the renal status glomerular filtration (GFR) rate was categorized as GFR < 60, GFR 60-89.9 and GFR $\geq$ 90.[5,7] A p-value of <0.05 was considered significant difference. Parametric tests were applied as majority of the continuous numerical data was found equally distributed and dispersed.

Results

In 154 study subjects the mean age was found similar in controls (55.7 years) and patients (56.5 years). Male gender was predominant and found equally distributed in patients and controls. (Table I).

The urinary albumin and creatinine was analyzed and it was found that there were 15 (19.4%) normoalbuminurics, 53 (68.8%) microalbuminuric and 9 (11.6%) cases with macroalbuminuria (Figure 1).

There was a gradual increasing trend of age and urinary albumin in the study subjects. The mean age of macroalbuminuric (59.3 $\pm$ 5.5 years) cases was significantly higher than controls (55.5 $\pm$ 5.1) and rest of albumin categories i.e. normoalbuminuric (56.2 $\pm$ 5.4) and microalbuminurics (56.5 $\pm$ 5.4). Male gender was predominant in the study and also in all albumin categories and controls, however, they were not

significantly different among categories (p-value, 0.58). (Table II).

Serum creatinine, serum cystatin C and other laboratory parameters were compared between albuminuria categories as well as controls. The levels of urine albumin (332.7 ± 30.1), creatinine (1.0 ± 0.13 mg/dl) and cystatin C (4.7 ± 3.9 mg/l) were found significantly high in the macroalbuminuria compared to controls and normoalbuminuric groups (p-value <0.001). Moreover, cystatin C was also found significantly associated with microalbuminuria than serum creatinine (2.6 ± 2.2 versus 0.94 ± 0.13 respectively).

GFR levels were significantly low in the micro (76.9 ± 15.1) and macroalbuminuria (74.0 ± 7.0) groups compared to normoalbuminuric and controls. Moreover, blood pressure was found significantly higher in the patients compared to controls (p-value <0.001). (Table III).

A selective analysis of cystatin C and serum creatinine levels was done according to GFR categories. The mean cystatin C was significantly high (1.7 ± 1.2) in patients with moderate to high kidney damage (GFR < 60), and mean cystatin C was also very high (2.6 ± 2.4) in patients with mild kidney damage (GFR 60-89). Serum creatinine was also found significantly deranged (1.2 ± 0.21) in GFR < 60 category, whereas in GFR 60-89 it was found border line deranged (0.94 ± 0.11). (Table IV).

Table I: Demographic, Clinical and Pathological Characteristics of Patients and Controls

Age (years)	Patients (n=77)	Controls (n=77)	p-value
40 to 50	19 (24.6%)	21 (27.2%)	0.91
51 to 60	41 (53.2%)	40 (51.9%)	
61 or above	17 (22.1%)	16 (20.7%)	
Mean ± SD	56.5 ± 5.5	55.7 ± 5.1	0.89
Gender			
Male	45 (58.4%)	45 (58.4%)	0.51
Female	32 (41.6%)	32 (41.6%)	
Laboratory Parameters			
Urine albumin	113.2 ± 106.8	5.2 ± 3.8	<0.001
Creatinine (mg/dl)	0.95 ± 0.14	0.56 ± 0.26	<0.001
Cystatin C (mg/l)	2.5 ± 1.9	0.45 ± 0.27	<0.001
GFR (ml/min/1.73m^2)	77.8 ± 14.5	125.1 ± 9.6	<0.001
Blood Pressure (mmHg)			
Systolic	148.0 ± 10.8	131.8 ± 8.2	<0.001

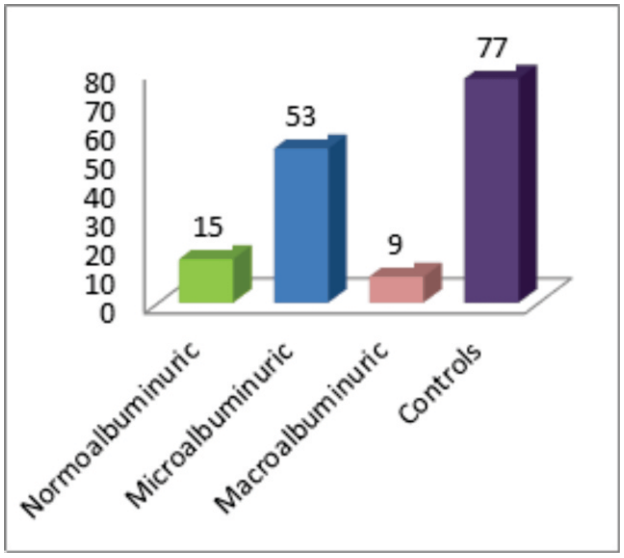

Fig 1: Distribution of Albuminuria in the Study Patients

Table II: Association of Age and Gender with Urine Albumin Status of Patients and Controls

Age (years)	Control (n=77)	Diabetic patients			p-value
		Normoalbuminurics (n=15)	Microalbuminurics (n=53)	Macroalbuminurics (n=9)	
Mean ± SD	55.7 ± 5.1	56.2 ± 5.9	56.5 ± 5.4	59.3 ± 5.5	<0.001
Gender					
Male	45 (58.4%)	8 (53.3%)	31 (58.5%)	6 (66.6%)	0.58
Female	32 (41.6%)	7 (46.7%)	22 (41.5%)	3 (33.3%)	

Table III: Comparison of Biochemical and Clinical Parameters between Controls and Albuminuria Categories

Parameters	Controls (n=77)	Normoalbuminuric (n=15)	p-value*	Microalbuminuric (n=53)	p-value*	Macroalbuminuric (n=9)	p-value*
Creatinine (mg/dl)	0.61 ± 0.21	0.69 ± 0.17	0.16	0.79 ± 0.38	0.701	1.0 ± 0.13	<0.001
Cystatin C (mg/l)	0.41 ± 0.29	0.56 ± 0.39	0.10	2.6 ± 2.2	<0.001	4.7 ± 3.9	<0.001
GFR ml/min/1.73m^2)	121.6 ± 9.1	83.1 ± 15.4	<0.001	76.9 ± 15.1	<0.001	74.0 ± 7.0	<0.001
HbA1c (%)	5.1 ± 2.8	7.1 ± 2.2	0.02	8.3 ± 2.7	0.01	8.9 ± 3.5	0.002
Systolic BP (mmHg)	130.6 ± 6.4	152.0 ± 9.0	<0.001	147.1 ± 11.1	<0.001	147.2 ± 11.5	<0.001

** p-values based on comparison of controls with individual albuminuric category*

Table IV: Relationship of Cystatin C and Creatinine with GFR Categories

Parameters	GFR < 60 (n=4)	GFR 60-89 (n=57)	GFR ≥ 90 (n=16)	p-value
Cystatin C	1.7 ± 1.2	2.6 ± 2.4	1.2 ± 0.9	<0.001
Creatinine	1.2 ± 0.21	0.94 ± 0.11	0.70 ± 0.18	<0.001

Discussion

The study findings reveal that cystatin C is significantly raised than creatinine in not only macroalbuminuria but also in cases of microalbuminuria. Microalbuminuria is most prevalent in the study, showing that two-third of patients were in the process of development of early diabetic nephropathy. Our study findings of raised cystatin C in early nephropathic derangement validate many previous reports on the topic. Lee BW witnessed that serum cystatin C is significantly lower in normoalbuminurics (0.83$\pm$ 0.22) than in microalbuminurics and macroalbuminurics (0.94 $\pm$ 0.33 and 1.05 $\pm$ 0.28 respectively; $p < 0.001$).[7] Jeon YK also witnessed a similar trend of relationship of cystatin C and diabetic nephropathy (micro and macroalbuminuria).[8,9,10]

Similarly in the current study the average serum creatinine and cystatin C are found high in micro and macroalbuminuric cases. Most of the study patients were in the early stage of diabetic nephropathy, however, 11.6% were proven cases of diabetic nephropathy (ACR > 300 mg/l). Cystatin C was found significantly high in micro and macroalbumin categories. Though serum creatinine was also found deranged in these cases, it is not that distinctive than cystatin C.

Previous literature on cystatin C suggests its superiority in detecting early diabetic nephropathy.[9,10,11] As patients on the risk of diabetic nephropathy can be recovered and early deterioration of renal function can be averted. This highlights the significance of an easy and feasible laboratory parameter like cystatin C.[4,9,12]

Serum cystatin C has proven its role as an alternative marker for estimating GFR. Moreover, the failure of creatinine to detect early decline in GFR is due to the fact that serum creatinine levels only start rising when almost 50% of renal function is lost, suggesting that GFR can change before serum creatinine becomes abnormal.[13,14] Cystatin C may rise faster than creatinine after a fall in GFR and is a reliable endogenous marker for assessing renal function in type 2 diabetic patients with renal impairment.[15,16]

It was found out that cystatin C and creatinine are significantly high in moderate to severe kidney damage and it is also high in mild kidney damage category (GFR 60-89). Our results have time and again proven that cystatin C was a highly useful marker of kidney damage in diabetic patients.

There are many advantages of the study which include; firstly, it was a comparative study comprising of diabetics and control groups, with a reasonable sample of seventy seven cases and seventy seven controls. Relationship of two commonly used markers i.e. cystatin C and serum creatinine were compared according to patient's albuminuria status and then also according to GFR status.

Conclusion

Based on the findings of current study it is concluded that cystatin C is a significant predictive marker of diabetic nephropathy than serum creatinine.

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