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A study of plasma Cystatin C as a marker of nephropathy in diabetes mellitus in a Tertiary Care Hospital

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Abstract
Diabetes mellitus continues to evolve as a global public health problem. Early identification of renal functions is important for diabetic patients. Serum Cystatin C may be one of the sensitive indicators of GFR. We in the present study tried to evaluate the usefulness and sensitivity of Cystatin C and creatinine in patients with diabetes mellitus type 2 in comparison with controls.
Methods: *The present study was conducted in the Departments of General Medicine and Biochemistry, ESIC Medical College and Hospital, Hyderabad from April 2016 to May 2018. A total of n=88 patients divided into two groups Group I (DM type II) n=44 and Group II (normal controls) n=44 were included in the study. The group I patients was subdivided into three subgroups based on the albuminuria. The subgroup [A] were the patients with microalbuminruia n=13, Subgroup [B] were patients with macroalbuminruia n= 17, Subgroup [C] were patients with normoalbuminuria n=14.*
Results: *In group I the mean age was 57.5 ± 3.5 years and the Mean age in the control group II was 55.5 ± 2.5 years. Cystatin C was more sensitive then creatinine in microalbumin subgroup [A] urea (73.3 and 75.1 % respectively) in Subgroup [B] the values were [63.5 and 69.8 % respectively] in normoalbuminuria subgroup [C] 59.5 and 54.6 % respectively While in control group serum creatinine was slightly more sensitive than the Cystatin C 42.3 and 41.5 % respectively.*
Conclusion: *it can be concluded that serum Cystatin C is a good marker of renal nephropathy and impaired renal functions in diabetic patients. In the study patients with macroalbuminuria had highest sensitivity with Cystatin C as compared to creatinine. It may be considered as an alternative marker of nephropathy instead of albuminuria in patients with decreased GFR and renal functions.*
Keywords: *Cystatin C, Diabetic nephropathy, creatinine.*

Introduction

Diabetes mellitus is a global health problem it is the combined result of an increase in life expectancy with a concomitant increase in access to refined carbohydrates and decrease in physical activity. Diabetes has reached epidemic proportions with current estimates of prevalence to about 170 million[1]. Chronic diabetes is associated with damage to several organs and dysfunctions especially with eyes, nerves, heart, kidneys and blood vessels[2]. The macrovascular complications include cardiomyopathy,

vasculopathy, dermatopathy and atherosclerosis [3]. Diabetic nephropathy is the leading cause of kidney replacement therapy and affects 40% of both type I and type II diabetic patients[4]. Diabetic nephropathy is defined by the presence of proteinuria in the absence of other renal diseases. It is more likely to occur in patients with poor glycemic control, hypertension, glomerular hyperfiltration and to an extent genetic predisposition. The early features of diabetic renal changes are microalbuminuria 30 - 300mg/day, glomerular hyperfiltration and renal hypertrophy with increased basement membrane thickness and mesangial expansion and accumulation of extracellular protein like collagen fibronectin and laminin[5]. Microalbuminruia precedes the development of macroalbuminruia and is predictive of future nephropathy. The onset of microalbuminuria in the absence of effective therapy usually leads to a gradual decline of GFR[6]. The Kidney Disease Improving Global Outcome KDIGO guidelines were the first to incorporate Cystatin C based formulas in addition to creatinine and GFR estimating formulae. Since creatinine is less accurate and can be affected by several factors including drugs[7]. Cystatin C is considered a good marker of kidney functions because it is solely filtered by the glomerulus and is not handled by renal tubules and is generated as a constant rate by cells of the body. Some studies have suggested that Cystatin C may detect a mild to moderate decrease in GFR which may not be evident with serum creatinine-based measurements. With this background, we in the present study tried to evaluate the sensitivity of Cystatin C as a marker of nephropathy in diabetic patients.

Material and Methods

The present study was conducted in the Departments of General Medicine and Biochemistry, ESIC Medical College and Hospital, Hyderabad from April 2016 to May 2018. Institutional Ethical committee permission was obtained for the study. Written consent was obtained for the study. The patients were grouped into two groups; Group I and Group II initially n=50 patients were included into both groups however 6 patients of the group I did not turned for follow up hence a total of n=44 patients were included similarly the group II patients were also reduced to n=44. The Group I was the known diabetic (Type II) patients who on treatment and group II patients were carefully selected normal patients without diabetes mellitus to act as age and sex-matched controls. Excluded patients were with other illnesses like malignancy, history of cardiovascular or renal diseases, history of hypertension, rheumatoid arthritis, and bronchial asthma. The group I patients was subdivided into three subgroups based on the albuminuria. The subgroup [A] were the patients with microalbuminruia (albumin excretion between 30 mg/day and 300 mg/day), subgroup [B] were patients with macroalbuminruia albumin excretion >300 mg/day subgroup [C] were patients with normoalbuminuria (albumin excretion in urine persistently lower than 30 mg/day). The anthropometric measurements and blood pressure were recorded. Blood samples were collected to determine FBS, HbA1c, serum uric acid, blood urea, serum creatinine. HbA1c was measured by chromatography based HPLC assay and expressed as a percentage. Serum creatinine was measured by Jaffe's method and serum Cystatin C was measured by ELISA. Microalbuminuria was determined by immunoturbidimetric method from urine collected over 24 hours expressed as mg/day. The results were recorded in MS Excel spreadsheet and analyzed by SPSS version 19 on windows format. A p-value of < 0.05 was considered as significant.

Results

The patients were grouped based on the age the range of the patients was from 40 to 65 years. In group I the mean age was 57.5 ± 3.5 years and the Mean age in the control group II was 55.5 ± 2.5 years. The majority of patients in the group I and group II belonged to age groups 56 to 65 years.

The distribution of patients in both groups is given in table 1.

Table 1: Age-wise distribution of cases in the study

Age group	Group I [Test]	Group II [Control]	Total	Percentage
40 – 45	3	3	6	6.81
46 – 50	9	7	16	18.18
51 – 55	7	8	15	17.05
56 – 60	15	14	29	32.96
> 60	10	12	22	25.00
Total	44	44	88	100.00

The group I patients were divided into three subgroups based on albumin urea. In microalbumin urea subgroup [A] total patients were n=13 and microalbuminuria subgroup [B] n=17 and normoalbuminuria subgroup [C] n=14. The total number of male in group I was n=28 (63.63%) and female were n=16 (36.36%) and in the control group II the male patients were n=30 (68.18%) and female patients were n=14 (31.81%) given in table 2.

Table 2: Sex wise and group-wise distribution of the patients in the study

	Group I			Total	Group II Control
	[A] Microalbuminuria	[B] Macroalbuminuria	[C] Normoalbuminuria		
Male	9	11	8	28	30
Female	4	6	6	16	14
Total	13	17	14	44	44

The duration of diabetes mellitus type II was recorded in all the patients, in normoalbuminuria subgroup [C] the duration of diabetes with the majority of patients was between 2 – 5 years and in the subgroup [B] the majority of patients where having diabetes from >10 years and in subgroup [A] majority of patients were having diabetes mellitus of duration greater than 6-10 years the other characteristics of the patients in the study are shown in table 3.

Table 3: Duration of diabetes mellitus in patients of Group I (test)

Duration	Group I			Total
	[A] Microalbuminuria	[B] Macroalbuminuria	[C] Normoalbuminuria	
2 - 5	1	2	10	13
6 – 10	8	6	4	18
> 10	4	9	0	13
Total	13	17	14	44

The values of BMI were recorded while in the control group the mean BMI was 30.85 ± 0.64 in the control group the mean values of BMI were 29.90 ± 1.10 Kg/m^2. The serum Cystatin C levels were measured in the groups the mean Cystatin C levels in Group I subgroup [B] was 3.15 mg/L and in subgroup [A] was 2.59 mg/L and in subgroup [C] was 1.51 mg/L. In the control group II the mean serum Cystatin C levels were 0.95 mg/L. The mean serum creatinine levels in group I was highest with subgroup [B] with mean values 2.05 mg/dl and lowest in subgroup [C] was 0.91 mg/dl. In group II the mean values of creatinine were 0.82 mg/dl. The other features are shown in table 4.

Table 4: values of parameters recorded in the group I and group II

Parameters	Group I			Group II Control	P values
	[A] Microalbuminuria	[B] Macroalbuminuria	[C] Normoalbuminuria		
BMI Kg/m^2	30.25 ± 1.25	31.55 ± 0.59	30.19 ± 0.70	29.90 ± 1.10	0.125
Serum Cystatin C mg/L	2.59 ± 0.20	3.15 ± 0.33	1.51 ± 0.29	0.95 ± 0.05	0.023*
Creatinine mg/dl	1.98 ± 0.32	2.05 ± 0.22	0.91 ± 0.23	0.82 ± 0.04	0.05*
* Significant					

The mean fasting blood glucose levels in the group I subgroup [A] was 159.5 mg/dl, in subgroup [B] values were 149.33 mg/dl and in Normoalbuminuria [C] values were 118.65 mg/dl. In the control group II the mean values of FBS were 89.95 mg/dl the p values were found to be significant. The HbA1c was highest in the Microalbuminuria subgroup [A] and lowest in Normoalbuminuria group [C]. The serum uric acid levels were highest in the microalbuminuria group and lowest in Normoalbuminuria group the p values were significant when compared with the control group.

Table 5: FBS, HbA1c, serum uric acid and blood urea recorded in the group I and group II

Parameters	Group I			Group II Control	P values
	[A] Microalbuminuria	[B] Macroalbuminuria	[C] Normoalbuminuria		
FBS [mg/dl]	159.5 10.5	149.33 9.8	118.65 8.5	89.95 3.3	0.02 *
HbA1c [%]	9.32 0.35	8.79 0.31	7.91 0.20	5.22 0.19	0.155
Serum Uric acid [mg/dl]	7.45 0.44	7.21 0.30	5.90 0.19	5.15 0.16	0.05*
B urea [mg/dl]	92.02 5.97	82.5 4.2	41.8 3.26	38.5 0.68	0.03*
* Significant					

A comparative study of serum Cystatin C and serum creatinine was done in group I and group II the Cystatin C was more sensitive than creatinine in microalbumin subgroup [A] urea (73.3 and 75.1 % respectively) in Subgroup [B] the values were [63.5 and 69.8 % respectively] in normoalbuminuria subgroup [C] 59.5 and 54.6 % respectively While in control group serum creatinine was slightly more sensitive than the Cystatin C 42.3 and 41.5 % respectively shown in table 6.

Table 6: Comparison of the sensitivity of Serum Cystatin C and serum creatinine

	Group I			Group II Control	P values
	[A] Microalbuminuria	[B] Macroalbuminuria	[C] Normoalbuminuria		
sensitivity of Serum Cystatin C	73.3	75.1	59.5	41.5	<0.001*
Sensitivity of Serum creatinine	63.5	69.8	54.6	42.3	0.04*
* Significant					

Discussion

Diabetes is marked by sustained hyperglycemia which results in changes in metabolic and hemodynamic functions[8]. Renal involvement is an important feature resulting in a decrease in renal functions over a period of time[9]. Diabetic nephropathy is an important cause of morbidity and mortality in type I and types II diabetes mellitus[10]. Hence, specific tests are necessary to monitor early signs of diabetic nephropathy to prevent the development of the end-stage renal disease. Earlier microalbuminuria with a decrease in creatinine clearance and an increase in serum creatinine was tested for diabetic nephropathy[11]. However, it has been shown that a decline in renal functions in patients with diabetes is not always accompanied by an increase of ACR[12]. Biomarkers for accurate estimation of renal function were searched for and one they were Cystatin C. Cystatin C is small protein MW 13kDa is a member of cysteine proteinase inhibitor family. It is produced constantly by all the nucleated cells of the body. Due to its small size, it is freely filtered by the glomerulus and is not secreted but is fully reabsorbed and broken down by the renal tubules. Cystatin C concentration was found to an endogenous marker of GFR and was considered superior to creatinine [11]. In the present study out of n=44 patients, n=30 (68.18%) were with diabetic nephropathy showing microalbuminuria and macroalbuminuria. The serum creatinine and

Cystatin C was found to be highest in patients with macroalbuminuria, as it is known that macroalbuminuria is the advanced stage of nephropathy. It was found in the study that patients with macroalbuminuria had diabetes with duration > 10 years which indicates progressive renal damage with chronic diabetes mellitus. In this study the sensitivity of serum Cystatin C was highest in the macroalbuminuria group at 75.1% followed by microalbuminuria group at 73.3% the similar values of the sensitivity of serum creatinine in macroalbuminuria group was 69.8% and microalbumin group was 63.5% and the p values were found to be highly significant in cystatin group. In a similar study by H J Waheed [5] in Iraqi patients found the serum Cystatin C in microalbuminuria group to be 76.2% and macroalbuminuria group to be 68.95% and the sensitivity of serum creatinine in microalbuminuria group 72.08% and macroalbuminuria group 61.33% agreeing with the results of the present study. The interesting finding of our study was that Cystatin C had a lower sensitivity to the normoalbuminuria group and the control patients. It must be then appropriate to consider that elevated Cystatin C levels when associated with microalbuminuria or macroalbuminuria is of clinical significance in diabetic patients. Studies have shown that Cystatin C now can be widely used and can replace creatinine for assessment of GFR in clinical practice. Roos et al; have concluded that Cystatin C is superior to serum creatinine as the marker of kidney function [13].

Conclusion

Within the limitations of the present study, it can be concluded that serum Cystatin C is a good marker of renal nephropathy and impaired renal functions in diabetic patients. In this study patients with macroalbuminuria had highest sensitivity with Cystatin C as compared to creatinine. It may be considered as an alternative marker of nephropathy instead of albuminuria in patients with decreased GFR and renal functions.

Conflict of Interest: None
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References

1. Shetty V, Jain HR, Singh G, Parekh S, Shetty S. Plasma Cystatin C as Marker of Early Renal Impairment in Diabetes Mellitus. Int J Sci Stud 2017; 4(12):1-7.
2. Choe JY, Park SH, Kim SK. Serum cystatin C is a potential endogenous marker for the estimation of renal function in male gout patients with renal impairment. J. Korean Med Sci 2010; 25: 42 48.
3. Al-Wakeel JS, Hammad D, Al Suwaida A, Mitwalli AH, Memon NA, Sulimani F. Microvascular and macrovascular complications in diabetic nephropathy patients referred to nephrology clinic. Saudi J Kidney Dis Transpl 2009; 20:77-85.
4. Jorge L. Gross, Mirela J. de Azevedo, Sandra P. Silveiro, Luís Henrique Canani, Maria, Luiza Caramori. 2005. diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care 2005; 28(1): 164 176.
5. Huda J Waheed. A Comparative Study for Cystatin C and Some Biochemical Markers for Predicting Diabetic Nephropathy in Iraqi Patients Int J Curr Microbiol App.Sci 2015; 4(3): 108-114.
6. I H de Boer, T C Rue, PA Cleary et al. Long-term renal outcomes of patients with type 1 diabetes mellitus and microalbuminuria: an analysis of the diabetes control and complications trial/epidemiology of diabetes interventions and complications cohort. Archives of Internal Medicine 2011; 171(5): 412–20.
7. KDIGO 2012 clinical practice guideline for evaluation and management of chronic

kidney disease. Kidney International Supplements2013; 3(1):1–150.

8. Schrijvers BF, De Vriese AS, Flyvbjerg A. From hyperglycemia to diabetic kidney disease: the role of metabolic, hemodynamic, intracellular factors and growth factors/cytokines. Endocrine Reviews. 2004; 25(6):971-10.
9. Toyota E, Ogasawara Y, Fujimoto K, Kajita T, Shigeto F, Asano T, et al. Global heterogeneity of glomerular volume distribution in early diabetic nephropathy. Kidney international. 2004; 66(2):855-61.
10. Rehman G, Khan SA, Hamayun M. Studies on diabetic nephropathy and secondary diseases in type 2 diabetes. Int J Diab Dev Ctries. 2005;25:25-29.
11. Shima T S, Khatun A, Yeasmin F. Cystatin C: A better predictor of kidney function in diabetic patients. Bangladesh J Med Biochem.2011; 4(1): 16 20.
12. Tsalamandris C. Allen T J, Gilbert R E, Sinha A, Panagiotopoulos S, Cooper M E, Jerums, G. Progressive decline in renal function in diabetic patients with and without albuminuria. Diabetes1994; 43: 649-55.
13. Roos JF, Doust J, Tett SE, Kirkpatrick CM. Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children - A meta-analysis. Clin Biochem 2007;40:383-91.