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SERUM CYSTATIN C AND MICROALBUMIN IN THE DETECTION OF EARLY NEPHROPATHY IN TYPE II DIABETIC PATIENTS.

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ABSTRACT

Early identification of impairment in renal function is crucial in diabetic patients. The aim of this study was to assess serum Cystatin C and Albumin Creatinine Ratio (ACR) in type II Diabetic patients. 50 diabetic patients of age 40-75years were included in this study. Fasting blood samples and morning urine samples were collected for analysis of glucose, urea, cystatin C, creatinine and microalbumin respectively. Statistical analysis was done using Medcalc. Patients were categorized into normoalbuminuric and microalbuminuric based on Albumin Creatinine Ratio (ACR). Cystatin C increased with increasing degree of microalbuminuria and correlated with ACR in the age group 40- 49 and 50-59. The level of Cystatin C was found to be higher in patients with GFR ≤ 60ml/min/1.732m^2 and hence Cystatin C is a predictor of early renal damage in patients even before the appearance of microalbuminuria. Therefore the determination of serum Cystatin C together with quantification of urinary microalbumin in patients with renal risk can optimize the early detection of renal damage.

KEY WORDS :Cystatin C; Creatinine; Microalbumin; Type II Diabetic Patients; Albumin Creatinine ration; Glomerular Filtration rate.

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INTRODUCTION

Diabetic Nephropathy is a significant complication in diabetic patients, and it is becoming the most common cause of ESRD (End stage Renal Disease) [1]. Therefore preventing diabetic nephropathy or delaying the disease progression by the way of early detection is very important. The determination of Microalbuminuria has been suggested as an early predictor of diabetic nephropathy [2]. The elevated urine albumin excretion within Microalbuminuric level (30 – 299 mg/g) allows the detection of patients with an increased risk for the development of overt nephropathy. Since the appearance of microalbuminuria is the first sign of nephropathy, patients with persistent microalbuminuria are referred to as having incipient nephropathy [3]. Creatinine clearance is a very good filtration marker in clinical practice but its accuracy is significantly hampered by assay interference, reliability of urine collection, influence of diet, age, gender and muscle mass [4] and there are several well reported difficulties concerning its analysis.[5,6]. Therefore the demonstration of Cystatin C as an early renal marker in diabetic patients was major step forward [7-9]. Serum Cystatin C is a cationic low molecular weight protein (13,359 KD) and is produced by all nucleated cells at a constant level [10-14]. It is freely filtered by the glomerulus and almost completely reabsorbed and degraded but not secreted by tubular cells. Serum Cystatin C have been claimed to be superior to creatinine as an endogenous marker of Glomerular filtration rate (GFR) as its Serum concentration is mainly determined by Glomerular filtration rate and not dependent on muscle mass or sex [15]. Coll.E et al has reported the presence of Cystatin C in patients with microalbuminuria but with a normal GFR and hence its measurement could be especially useful in detection of early nephropathy [16]. Serum Cystatin C has also been reported to have advantage over serum creatinine in monitoring nephropathy in diabetic patients [17].

AIM

The aim of the study was to assess the early kidney damage in the patients with type II diabetes mellitus through the quantification of Serum Cystatin C and ACR and to evaluate the relation between serum Cystatin and ACR.

METHODS

50 known diabetic patients with age ranging from 40 to 75 were included in this study. The study was approved by the institutional ethical committee of Madras Medical College and informed consent was taken from all the patients. The demographic data such as age, sex, duration of Diabetes mellitus, family history of Diabetes Mellitus, renal disease were recorded for each patient. Height, weight, waist, hip measurements were taken and Body mass index (BMI) and Waist Hip ratio (W/H ratio) was calculated. Fasting venous blood was collected for measuring serum creatinine, glucose, Cystatin c and urea. Morning urine sample was collected in container for analysis of creatinine and Microalbumin. Creatinine was analyzed by Jaffes method, Microalbumin by immunoturbidometric method using spine react kit (Spain). Serum Cystatin c was measured using particle enhanced nephlometric immnuno assay.

GFR was calculated using MDRD formula.

GFR (ml/min) for men = 186* (serum creatinine in mg/dl) $^{-1.54}$*age$^{-0.203}$

GFR (ml/min) for women = 186* (serum creatinine in mg/dl) $^{-1.54}$*age$^{-0.203}$ *0. 742

Cystatin C was considered high, according to the recommendation of the manufacturer with a reference range of 0.55-1.15mglL for individuals up to 50 years and 0.63-1.44mg/L for individuals above 50 years

STATISTICS

Statistical Significance among the groups was determined using the Medcalc 8.1 Statistical Software (Belgium). Descriptive analysis was performed using the mean and Standard deviation. The correlation between the variables were evaluated using Pearson's Correlation coefficient after which step wise multiple linear regression was performed with variables to find out the factor that affects Cystatin C, Serum Creatinine and ACR. All the results were considered significant if $P<0.05$. Comparison of mean between the variables with two categories was performed using student t test.

RESULTS

TABLE I
BASELINE AND CLINICAL CHARACTERISTICS OF DIABETIC PATIENTS

Parameters	Diabetic Patients with normal micro albumin(<30mg/g)	Diabetic Patients with Micro albumin (30-299mg/g)	P value
Number	12	38	
Age (years)	54.75±8.21	56.73±9.09	0.48
Duration of diabetes(years)	9.67±4.46	11.5±5.23	0.25
BMI(kg/m^2)	26.24±4.11	27.07±4.20	0.55
Waist Hip ratio	0.900±0.04	1.08±1.28	0.39
Systolic BP(mmHg)	125±10.87	122.89±9.56	0.57
Diastolic BP(mmHg)	79±6.23	82.47±6.24	0.11
Glucose(mg/dl)	169.58±34.41	163.84±38.95	0.63
Urea(mg/dl)	22.5±7.89	27.34±8.52	0.08
Serum creatinine(mg/dl)	1.07±0.31	1.05±0.34	0.93
Urine creatinine(mg/dl)	1.17±0.62	0.93±0.35	0.21
ACR (mg/g)	16.06±5.044	79.85±47.50	0.00**
Cystatin c(mg/l)	1.19±0.62	1.738±0.661	0.02*
eGFR (ml/min/1.732m^2)	66.16±32.02	68.07±22.86	0.75

BMI: Body mass index, ACR: Albumin Creatinine Ratio, eGFR: Estimated Glomerular filtration rate.
**** P value is significant.***
***** P value is highly significant***

The Baseline characteristics of the subjects are shown in Table1. Subjects were classified as normoalbuminuric (ACR values less than 30) and microalbuminuric (ACR between 30-300mg/g). There is no significant difference in age, Serum creatinine, duration and BMI between the two groups. However Cystatin C and ACR level were significantly higher in Microalbuminuric group (1.74±0.66 and 79.85±47.5) respectively than normoalbuminuric group (1.19±0.62 , 16.06±5.044).The p value was found to be <0.05.

TABLE 2
Biochemical data for the patients based on their age groups

	AGE		
	Group 1	Group 2	Group 3
Parameters	40-49 yrs(n=13)	50-59yrs(n=16)	>60yrsn=(21)
Age (years)	45±3.188	55.32±1.8	64.71±4.48
Duration of diabetes(years)	9.53±2.47	8.85±2.96	13.42±6.4
BMI(kg/m^2)	27.86±3.26	26.41±4.23	27.05±4.7
Waist Hip ratio	0.879±0.07	0.90±0.04	1.25±1.74
Systolic BP(mmHg)	121.53±8.0	123.07±11.82	124.76±9.8
Diastolic BP(mmHg)	82.46±5.95	82.77±7.28	79.61±5.91
Glucose(mg/dl)	174.61±35.33	175.77±39.24	152.67±38.19
Urea(mg/dl)	25.46±4.31	23.23±6.27	27.38±9.68
Serum creatinine(mg/dl)	0.99±0.24	1.13±0.3	1.06±0.41
Urine creatinine(mg/dl)	1.15±0.65	1.02±0.313	0.89±0.36
ACR (mg/g)	50.15±36.74	57.11±35.5	80.47±62.91
Cystatin C(mg/l)	1.31±0.60	1.76±0.60	1.75±0.78
eGFR(ml/min/1.732m2)	71.92±20.32	60±19.01	68.19±23.8

BMI: Body mass index, ACR: Albumin Creatinine Ratio, eGFR: Estimated Glomerular filtration rate. * P value is significant. ** P value is highly significant. In table 2, the patients were categorized in to three groups based osn age as group 1 (40-49 yrs n=13), group 2 (50- 59 yrs n=16), group 3(>60 yrs n=21).

In group 1, the stepwise multiple regression analysis between Cystatin C and ACR showed significant correlation with ACR (r=0.7433; p<0.05) than Serum Creatinine (r=-0.017; p=0.9550).That is increased level of cystatin C was always accompanied by an increase in ACR, where as the increased level of cystatin C was not accompanied by increase in serum creatinine. However Serum Creatinine correlates well with eGFR(r=-0.7842; p=0.0015) than Cystatin C (r=0.0619; p=0.8406) and ACR (r=0.064; p=0.8353) . Likewise, in group 2, the step wise multiple regression analysis between Cystatin c and ACR showed significant correlation (p=0.04; r=0.5089) than Serum creatinine with ACR (r=-0.0908; p=0.7379. Serum creatinine correlates well with eGFR (r=-0.8635; p<0.0001) In group 3, the step wise multiple regression analysis between Cystatin C and ACR did not show any significant correlation (r=-0.2004;p=0.3837). However Cystatin C showed a good correlation with Serum creatinine (p=0.0234;r=0.4922) . As the age increases there is corresponding elevation of serum creatinine and Cystatin C. Serum creatinine also showed good correlation with eGFR and number of years (r=0.7199;p=0.0002), (p=0.0146;r=0.5247) in this group.

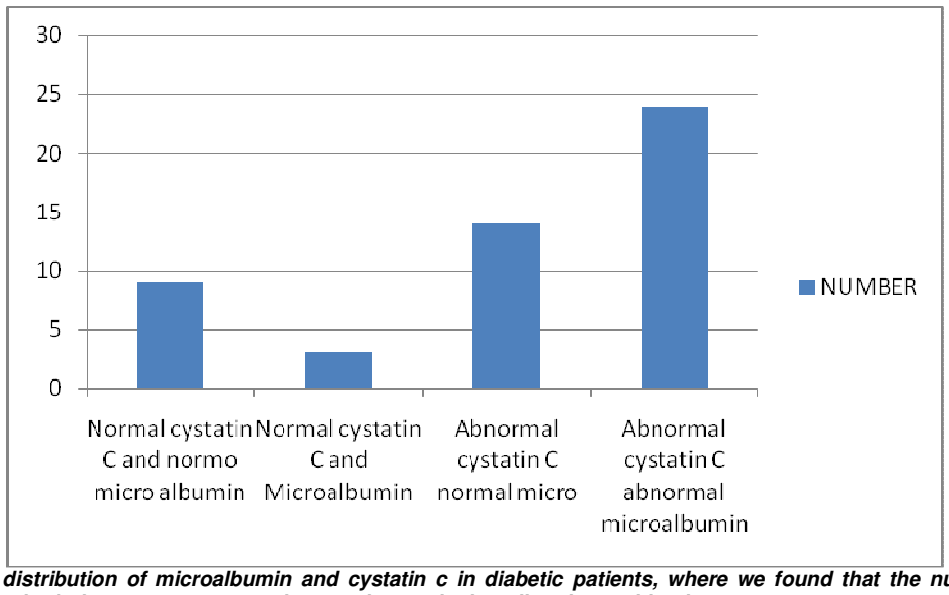
TABLE 3
Characteristics of diabetic patients defined by using eGFR (ml/min/1.732m2) calculated by MDRD equation:

arameters	eGFR≤60 ml/min/1.732m^2	eGFR>60 ml/min/1.732m^2	p value
	(n=21)	(n=29)	
Age (years)	57.76±8.59	55.17±9.06	0.31
Duration of diabetes(years)	10.9±6.04	11.17±4.38	0.86
BMI(kg/m^2)	27.3±4.65	26.5±3.73	0.00**
Waist Hip ratio	0.899±0.06	1.14±1.50	0.40
Systolic BP(mmHg)	122.86±10.07	123.79±9.89	0.74
Diastolic BP(mmHg)	82.23±6.82	81.20±6.18	0.58
Glucose(mg/dl)	163.38±43.27	166.55±34.38	0.78
Urea(mg/dl)	27.38±9.92	25.31±7.6	0.43
Serum creatinine(mg/dl)	1.3±0.37	0.88±0.15	0.00**
Urine creatinine(mg/dl)	0.87±0.33	1.07±0.47	0.09
ACR (mg/g)	70.27±57.06	60.38±44.95	0.51
Cystatin c(mg/l)	1.88±0.78	1.40±0.55	0.02*
eGFR (ml/min/1.732m2)	49.95±9.22	80.41±14.92	0.00**

BMI: Body mass index, ACR: Albumin Creatinine Ratio, eGFR: Estimated Glomerular filtration rate * P value is significant.
***** p value highly significant***

The diabetic patients were grouped based on GFR (table: 3) as group 1 (GFR less than 60) and group 2 (GFR more than 60). We found that there exist statistical significance between mean values of BMI, serum creatinine, Cystatin C and eGFR equation between the two groups. In group1 ACR correlated with duration of diabetes and BMI (r=0.6184; p=0.002) and (r=0.0145, p=0.01) respectively. Cystatin correlated with BMI (p=0.01; r=0.5251). Serum creatinine correlated with eGFR, duration of diabetes. In group 2 ACR also correlated with age (r=0.3725; p=0.04) and duration of diabetes (r=0.4517; p=0.01).Cystatin C correlated with age(r=0.3755;p=0.04) and BMI (r=0.5251;p=0.01). Serum creatinine negatively correlated with eGFR (r=-0.6934) and BMI (r=-0.5431), P value is less than 0.03

Figure: III
Distribution of microalbumin and cystatin C in Diabetic patients.



The fig III, shows the distribution of microalbumin and cystatin c in diabetic patients, where we found that the number of nephropathic patients increased when both the parameters namely cystatin c and microalbumin combined.

DISCUSSION

In this study, we aimed at identifying early nephropathy changes in type II diabetic patients. Patients were categorized based on ACR level. Cystatin were found to be higher in microalbuminuric patients and patients with GFR≤60/mi/min/1.732m^2. The routine evaluation of diabetic nephropathy includes the detection of Microalbumin, decreased GFR and increased serum creatinine [18]. But it has been reported that decline in the renal function of patients with diabetes was not always accompanied by an increase ACR [19]. About 20-30 percent of patients with type II diabetes, accompanied by renal insufficiency showed normoalbuminuric [20]. Therefore other biomarkers for estimation of renal function have been searched and one of them was Cystatin C. In our study we evaluated the results of both Cystatin C and Microalbumin in diabetic patients and found that both the level increased in diabetic patients. Some authors have established a relationship between increased Cystatin C and subsequent appearance of microalbuminuria [21]. Cystatin C correlated with BMI in patients with GFR ≤60ml/min/1.732 m^2 which is in agreement with Knight et al who reported that serum Cystatin C level are influenced by multiple factors including greater height and weight [22,23] .Various authors' reported increased Cystatin C values with advancing age [24] which is similar to our study. Our previous study has shown that the mean duration of diabetes was significantly longer for patients with microalbuminuria compared with normal microalbuminuria [3] which is in accordance with this study.

CONCLUSION

In our study, we have found that Microalbuminuria is significantly correlated with cys c indicating that cys c is a sensitive marker of incipient Nephropathy. It is a valuable tool to describe GFR loss independently and together with ACR among the patients with diabetes.

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