

A STUDY OF SERUM CYSTATIN-C AND ITS CORRELATION WITH MICROALBUMINURIA AS MARKER FOR DIABETIC NEPHROPATHY IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Diabetic nephropathy is the single most frequent cause of end stage renal disease. GFR is the best index of renal function in health and disease. Its direct measurement with inulin or EDTA requires specialized technical personnel. Properties of an ideal endogenous blood substance to estimate GFR should include release into blood stream at constant rate and free filtration by glomerulus. Serum Creatinine is the most commonly used filtration marker in clinical practice but its accuracy is significantly hampered by the confounding influence of diet, age, gender and muscle mass. Microalbuminuria is first detectable functional abnormality, but there is 40% day to day variability. Several factors can increase urinary albumin like exercise, UTI, CCF. Cystatin C is a 13kDa protein expressed in all nucleated cells and produced at constant rate. It is freely filtered by glomerulus. It does not return to blood stream and is not secreted by renal tubules. It has been suggested to be "ideal" endogenous marker.

Aims and objectives:

- ❖ To estimate serum cystatin C levels and microalbuminuria in diabetic patients.
- ❖ To compare serum cystatin C levels and microalbuminuria in diabetic patients.

Methodology: One hundred patients with diabetes were included in study. Data was collected through a prepared proforma which included various parameters related to history, thorough clinical examination, diagnosis and laboratory parameters. All the Patients were subjected to serum creatinine, cystatin C and urine albumin to creatinine ratio along with other relevant laboratory investigations.

Results: This study demonstrates majority of patients were in 41 to 50 years age group (39%), predominantly males. There was a positive correlation between duration of diabetes and creatinine levels, microalbuminuria (Correlation coefficient – 0.548, P <0.05), and serum cystatin C levels (Correlation coefficient - .545, P <0.05). There was significant correlation between Cystatin C, creatinine levels (Correlation coefficient - .683, P <0.05) and microalbuminuria (Correlation coefficient - .593, P <0.05).

Conclusion: A higher percentage of subjects had normoalbuminuria. More subjects in the proteinuric groups were associated with co-morbid condition and complication of diabetes and also had poorer glycemic control. Most of the subjects who presented early had normal serum creatinine, normoalbuminuria with normal to slightly decreased GFR. Cystatin C levels were elevated in few of them. However, most subjects with elevated cystatin C had normal serum creatinine levels, normoalbuminuria and reduced GFR. Serum Cystatin C may be considered as an early marker, than both microalbuminuria and serum creatinine.

Key words: Cystatin C, Microalbuminuria, Creatinine, eGFR, Diabetic Nephropathy.

INTRODUCTION

Diabetes Mellitus (DM) comprises a common group of metabolic disorders that share the phenotype of hyperglycemia and are caused by complex interaction of genetics, environmental factors and life style choices^{2,3,4}.

The number of people with diabetes in world is expected to approximately double between 2000 and 2030 from 2.8% to 4.4% (171 million to 366 million). The greatest absolute increase in the number of people with diabetes will be in India. WHO estimates a projected rise to 80 million diabetics in India by 2030 from 32 million in 2000. Type 2 Diabetes mellitus accounts for 90-95% of all diabetics in India⁵⁻⁷. The burden of the disease and its complications is increasing and hence there is greater need to recognize and manage the disease at earliest.

India needs to implement the preventive measures to reduce the burden of diabetics as it poses a medical challenge, which is not matched by budget allocated for diabetes care in India. It is estimated that the annual cost of diabetes care were approximately Rs 90,200 million. The average expenditure per patient per year is about a minimum of Rs 4,500⁶.

Diabetic nephropathy is the leading cause of Diabetes Mellitus related mortality and morbidity. Its prevalence in India varies between 5-9% in many studies⁹. It is also one of the leading causes of chronic renal failure in India⁹.

The most important measure of diabetic nephropathy is Glomerular Filtration Rate (GFR) which is an index of renal function. Renal Function is evaluated commonly by estimation of

- (a) Serum Creatinine (sCr) and Blood Urea
- (b) Urinary microalbuminuria and Albumin Creatinine ratio (ACR)

However these parameters are affected by multiple factors and hence not very reliable¹⁰.

Creatinine Clearance rate has also been used to estimate Glomerular Filtration Rate. However its reliability is greatly diminished by variability in renal

tubular secretion of creatinine and by the inability of most patients to accurately collect timed urine samples. It also does not improve the estimation of Glomerular Filtration Rate over that provided by prediction equation of Glomerular Filtration Rate estimation by urinary clearance of Inulin, Iothalate, CrEDTA which are accurate, are expensive and are not readily available.

Microalbuminuria is first detectable functional abnormality, but there is 40% day to day variability. Several factors can increase urinary albumin like exercise, UTI, CCF. Tests with positive results should be repeated and a patient is considered to have elevated urinary albuminuria if 2 out of 3 results are abnormal within 3 months frame¹¹. Cystatin C is a 13kDa protein expressed in all nucleated cells and produced at constant rate. It is freely filtered by glomerulus. It does not return to blood stream and is not secreted by renal tubules. It has been suggested to be "ideal" endogenous marker¹².

There are not many studies from India comparing Cystatin C, Creatinine levels and microalbuminuria in diabetic subjects. Hence this study was conducted to look at Cystatin C, as a marker of declining renal function in comparison with the other parameters in Type 2 Diabetes Mellitus patients.

MATERIAL AND METHODS

Source of data

The study comprised of patients with diabetes, both inpatients and out patients in the department of medicine, Victoria Hospital and Bowring and Lady Curzon Hospital, Bangalore Medical College and Research Institute.

Method of collection of data

- a) Sample size – 100
- b) Type of study – A prospective comparative nonrandomized study
- c) Duration of study – 2 yrs
- d) Method – A total of 100 cases with diabetes and above age 18 yrs were recruited for the study, based on inclusion and exclusion criteria. Informed consent was taken from all

subjects. Ethical clearance was taken from the ethical committee of the institution. Each individual was evaluated historically and by clinical examination for collection of clinical data by a proforma designed for the study with special reference to baseline characteristics, duration of diabetes, diabetic complications and confounding factors. Each subject was then subjected to investigations as stated below.

Inclusion criteria

Patients more than 18 yrs old with diabetes, either known, on or off treatment, or newly diagnosed as per ADA criteria.

Patients giving written informed consent

Exclusion criteria

Subjects with the following disorders were not included in the study – hypothyroid or hyperthyroid patient, pre-existing renal disease, critically ill patients, post renal transplant, patients on steroids or cyclosporine, urinary tract infections, congestive cardiac failure, with active menstruation, chronic liver disorders. Patients with gross proteinuria and who were already on ACE inhibitor therapy were excluded from the study.

Sample procedure

Clinical history

- ❖ Detail history was taken regarding the duration of diabetes, symptoms of hyperglycemia (polyuria, polydipsia, polyphagia, weight loss etc) and symptoms suggestive of diabetic complications
- ❖ Treatment History of diabetes was taken
- ❖ History was taken to exclude any acute infections or illness and the conditions mentioned under exclusion criteria that might interfere with the results.
- ❖ Family History of diabetes mellitus and its complications was taken
- ❖ History of smoking and alcohol intake was taken

Examination

Routine physical examination was done and anthropometric measurement of height, weight and Body Mass Index (BMI) were calculated in all patients. Vital data (like BP, Pulse etc) of each patient was recorded as per the protocol. Systemic examination was done in detail for the evidence of following complications

1. Detection of Diabetic Peripheral neuropathy by
 - a) Deep tendon reflex testing by percussion hammer
 - b) Vibration perception testing by 128 Hz tuning fork
2. Detection of Diabetic Retinopathy by Direct ophthalmoscopic examination of fundus – results was classified as normal, background retinopathy (BGR), non-proliferative (NPDR) and Proliferative diabetic retinopathy (PDR).
3. Detection of Diabetic nephropathy was investigation based, by determination of Urinary Albumin-Creatinine ratio (ACR).

Investigations

1. Estimation of Hemoglobin, Total count and Differential count was done by autoanalyser, sysmao (k-100)
2. Blood sugar levels were estimated in all patients using autoanalyser technique RA-XT. FBS and PPBS were done for the diagnosis of diabetes based on ADA criteria and expressed as mg/dl.
3. HbA1C – Glycosylated hemoglobin was quantitatively determined by immunoturbidometry. A value of > 6.5 gm/dl was taken as evidence of poor glycemic control
4. Renal function test – Blood urea and serum creatinine was done by autoanalyser technicon – RA-XT. Serum creatinine was estimated by Jaffe's method and expressed as mg/dl. The upper limit of normal was 1.2 mg/dl
5. Routine examination of urine for protein, sugar and microscopy was done in all subjects. Those

with abnormal microscopy were not taken to exclude anyone with possible urinary tract infection or primary glomerular disease.

6. Urinary Albumin to Creatinine ratio (expressed in $\mu\text{g}/\text{mg}$) estimation was done. Urinary albumin was measured by rate nephelometry and urinary creatinine was measured by modified Jaffe's method.
7. USG abdomen was done to look for evidence of nephropathy
8. ECG was done in all patients and 2D-ECHO in few (where ECG showed changes) to look for any evidence of cardiac dysfunction
9. Serum Cystatin C was measured by particle enhanced nephelometric immunoassay (PENIA) method and expressed as mg/l. The normal range being 0.53 – 0.95 mg/l.

Body Mass Index (BMI)

BMI was calculated from the formula $\text{BMI} = \text{weight in kg} / \text{height in cm}^2$. Subjects were classified as

- a) Under-nourished < 18.5
- b) Normal 18.5-25
- c) Overweight 25-30
- d) Obese >30

Glomerular Filtration Rate (GFR)

GFR was determined by using the Cockcroft-Gault formula (C-G)

$\text{CrCl (ml/min)} = (140 - \text{Age}) \times \text{Wt (kg)} (0.85 \text{ to be multiplied in females})$

$$72 \times P_{\text{Cr}}$$

where P_{Cr} stands for plasma creatinine and CrCl for creatinine clearance

This method was used as per the National Kidney Foundation guidelines¹³ that recommends calculated GFR to determine renal function. Other methods to determine GFR like timed urine sample or radioisotope methods were not considered, as they were cumbersome, expensive and not easily available.

The subjects were then classified as per the criteria for chronic kidney disease (CKD) staging into

Stage 1 (>90 ml/min)

Stage 2 (60-89 ml/min)

Stage 3 (<60 ml/min)

Analysis of Data

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data are made, **Assumptions:** 1. Dependent variables should be normally distributed, 2. Samples drawn from the population should be random, and Cases of the samples should be independent.

Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients, Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Significant figures

+ Suggestive significance (P value: $0.05 < P < 0.10$)

* Moderately significant (P value: $0.01 < P < 0.05$)

** Strongly significant (P value: $P < 0.01$).

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS

In this study 100 subjects with diabetes were evaluated. Serum Cystatin C levels and ACR were studied in comparison with other conventional variables

The study had a male dominance which comprised 56% of the total population. 44% populations were

females. In this study most of the cases were in the age group of 41-50 yrs which comprised 39% of the total population. Mean age group among the cases was 51.6 yrs.

Duration of Diabetes

In our study majority of the patients (56%) had duration of diabetes less than 5 yrs. Only one patient had for more than 15 yrs. Median of duration was 5 yrs. Elderly people had increased duration of diabetes. There was a positive correlation between duration of diabetes and age. The study had a male dominance. There was no statistical difference between sex of the subject and duration of diabetes. There was a positive correlation between duration of diabetes and creatinine levels with increase in creatinine with duration. Statistically it was significant ($p < 0.05$). With increase in duration of diabetes, more subjects had microalbuminuria. There was a positive correlation and statistically it was significant. (Correlation coefficient – 0.548, $P < 0.05$)

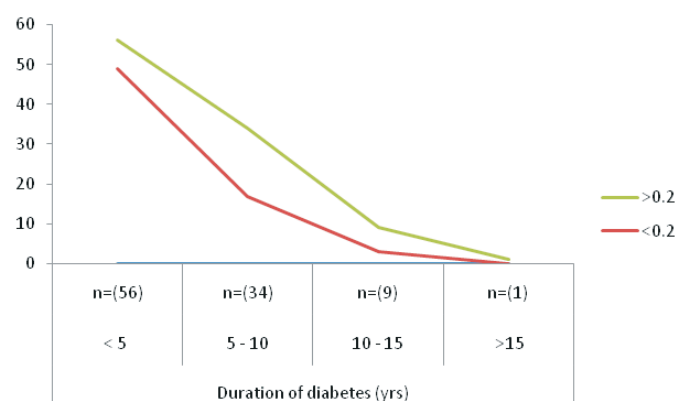


Figure 18 - Correlation between duration of diabetes and albuminuria

| | Duration of diabetes (yrs) | | | | Total |
|------------|----------------------------|--------|---------|-------|-------|
| | < 5 | 5 - 10 | 10 - 15 | >15 | |
| | n=(56) | n=(34) | n=(9) | n=(1) | |
| Cystatin C | | | | | |
| < 0.95 | 46 | 12 | 1 | 1 | 60 |
| > 0.95 | 10 | 22 | 8 | 0 | 40 |

Table 10 - Correlation between duration of diabetes and Cystatin C levels

There was a positive correlation between Cystatin C levels and duration of diabetes. Statistically it was significant. (Correlation coefficient - .545, $P < 0.05$).

| eGFR | Duration of diabetes (yrs) | | | | Total |
|-------|----------------------------|--------|---------|-----|-------|
| | < 5 | 5 - 10 | 10 - 15 | >15 | |
| >90 | 30 | 3 | 0 | 0 | 33 |
| 60-90 | 25 | 22 | 3 | 1 | 51 |
| <60 | 1 | 9 | 6 | 0 | 16 |

Table 11 - Correlation between duration of diabetes and eGFR

There was a positive correlation between duration of diabetes and eGFR values. Statistically it was significant. ($P < 0.05$)

Blood Pressure

There was no significant difference noted in the mean systolic and diastolic blood pressure values between the patients with elevated Cystatin C levels and normal levels. Also there was no significant difference in blood pressure in patients with microalbuminuria and normalalbuminuria.

The mean systolic BP in our study was 130.56 mm Hg with a mean diastolic BP of 80.21 mm Hg.

Body Mass Index

The mean BMI was 26.74. There was no statistical difference in BMI between patients with elevated Cystatin C and normal Cystatin C. There was no statistical difference in BMI between patients with normalalbuminuria and microalbuminuria.

57% of the patients in our study were either overweight or obese with only 42% having normal BMI. There was a positive correlation between BMI and microalbuminuria. It was statistically significant.

There was a positive correlation between BMI and Cystatin C levels, however statistically it was not significant.

Blood sugars

The mean FBS among diabetics was 183 ± 25.83

mg/dl. The mean PPBS among diabetics was 254 ± 32.87 mg/dl. There was a positive correlation between sugar levels, microalbuminuria and Cystatin C level. However, it was not statistically significant.

Glycosylated Hemoglobin

The mean HbA1C level in our study was 9 ± 1.54 %. There was a positive correlation between HbA1C, microalbuminuria and Cystatin C level. However, it was not statistically significant.

Serum Creatinine (mg/dl)

The mean creatinine level in study was $0.954 \pm .34$ mg/dl. 18% of the patients had elevated creatinine levels.

Fundus changes

23% of the total patients had fundus changes.

Peripheral Neuropathy

10% of the total population had some signs or symptoms of neuropathy.

Ultrasound changes

18 % of the total population had grade 1 medical renal disease.

Albumin to Creatinine ratio

31% of the patients in our study had microalbuminuria.

There was a positive correlation between ACR and Creatinine levels. Statistically it was significant ($P = < 0.056$). Correlation coefficient - .584

There was a negative correlation between eGFR and ACR. It was statistically significant with correlation significance of -.684 and P value < 0.01

Serum Cystatin C

The normal range for Cystatin C is 0.53-0.95 ng/ml. In our study the median value for Cystatin C was .87. 40% of the subjects had elevated Cystatin C level.

There was a positive correlation between Cystatin C and ACR. It is statistically significant with p value of < 0.01 . The correlation coefficient being .593

There was a positive correlation between Cystatin c level and creatinine values. Statistically the p value is < 0.01 . The correlation coefficient being .683

There was a negative correlation between eGFR and Cystatin C. However statistically it was significant ($P < 0.01$). The correlation coefficient being -.726

There was a positive correlation with ultrasound abdomen and Cystatin C as well as Fundus and neuropathy with Cystatin C which is statistically significant.

DISCUSSION

Diabetes Mellitus comprises a common group of metabolic disorders that share the phenotype of hyperglycemia and are caused by complex interaction of genetic, environmental factors and life style choices.

Diabetic nephropathy is the single most frequent cause of end stage renal disease. GFR is the best index of renal function in health and disease. Its direct measurement with inulin or EDTA requires specialized technical personnel. Properties of an ideal endogenous blood substance to estimate GFR should include release into blood stream at constant rate and free filtration by glomerulus. Serum Creatinine is the most commonly used filtration marker in clinical practice but its accuracy is significantly hampered by the confounding influence of diet, age, gender and muscle mass.

Microalbuminuria is first detectable functional abnormality, but there is 40% day to day variability. Several factors can increase urinary albumin like exercise, UTI, CCF. Tests with positive results should be repeated and a patient is considered to have elevated urinary albuminuria if 2 out of 3 results are abnormal within 3 months frame

Cystatin C is a 13kDa protein expressed in all nucleated cells and produced at constant rate. It is freely filtered by glomerulus. It does not return to blood stream and is not secreted by renal tubules. It has been suggested to be "ideal" endogenous marker.

The present study was conducted to look at Cystatin C, as an earlier and better marker of declining renal function, in comparison with the commonly used parameters in diabetes mellitus patients. It was done in 100 diabetes patients presenting in the

department of Medicine, Bangalore Medical College and Research Institute at either Victoria Hospital or Bowring and Lady Curzon Hospital.

Baseline Characteristics

1. 65% of the subjects were asymptomatic. 20% were symptomatic for hyperglycemia and 19% symptomatic for diabetic complication. 50% of patients with microalbuminuria presented with symptoms due to complication.
2. 16% subjects were on treatment with insulin therapy only. 9 subjects were on treatment with both insulin and oral hypoglycemic agent of which 8 had microalbuminuria.
3. Most patients with history of hypertension, dyslipidemia, smoking and alcohol had microalbuminuria.
4. 23% had abnormal findings on fundoscopy, 14 had BDR, 8 had NPDR and 1 had PDR. All 23 patients had microalbuminuria.
5. 10 had neuropathy detected on vibration testing. 9 of them had microalbuminuria.
6. 18 had nephropathy detected on USG. 14 of them had microalbuminuria.

The above findings reiterate that proteinuria is associated with other comorbid conditions and an increased incidence of complications, reflecting the fact that it is a marker of widespread endothelial dysfunction and cardiac dysfunction.

Age distribution

In the present study the mean age of presentation of all diabetic subjects was 51 yrs. The highest incidence of diabetes was noted in the age group of 41-50 (39%). This is due to the fact that more subjects with diabetes in its initial stages of diagnosis were taken, as required by the study.

It was seen that with age, the incidence of microalbuminuria also increased. This can be explained by the fact that as the age advances, presentation of DM increases, and proteinuria also increases.

Sex distribution

56% of the subjects were male and 44 % were female

Our study shows that lesser percentage of females presented with proteinuria. This can be explained partly due to exclusion of subjects with gross proteinuria and that the females presented earlier than males with mean duration of 4.4 yrs as compared to 5.75 for males.

Duration of diabetes

In present study the mean duration of diabetes was 5 ± 3.4 which is correlated with the study conducted by Yang YS et al⁷². However it did not match the findings of other studies. An explanation for this would be that diabetes with small duration only was taken in our study whereas in other studies, patients were taken with duration of diabetes beyond 30 yrs also.

56% of the subjects had diabetes of <5 years duration. This is due to the fact that more subjects with DM in the initial period of diagnosis were recruited for the study, as was required by the study.

Blood pressure (mm/Hg)

In the present study the mean SBP was 130.56 and mean DBP was 80.5. These values were lower than other studies.

Body Mass index (kg/m²)

The mean BMI was 26.74 ± 3.45 . These were in comparison with other studies by Yang YS et al⁷² and Vishwanathan V et al⁷¹. Perkins BA et al⁷⁵ study had higher BMI of 33 ± 7

HbA1C level

In our study the mean HbA1C was 9 ± 1.54 . This was in comparison to other studies like that of Vishwanathan V et al.⁷¹

Serum Creatinine (mg/dl)

The mean serum creatinine was $0.954 \pm .34$ mg/dl. As expected serum creatinine levels significantly increased in microalbuminuria group ($p < 0.001$). This correlated well with other study by Yang YS et al⁷² and Vishwanathan V et al⁷¹

Serum Cystatin C

The mean serum Cystatin C levels in our study was 0.96 ± 0.29 . Out of the 100 subjects, 40 had serum Cystatin C levels above the upper limit of normal (>0.95 mg/l). The results correlated with the studies by Yang YS et al.⁷²

| | Yang YS et al study ⁷² | Piwowar A et al study ⁷³ | Christenson AG et al ⁷⁴ | Perkins BA et al ⁷⁵ | Vishwanathan V et al ⁷¹ | Present study |
|------------------|-----------------------------------|-------------------------------------|------------------------------------|--------------------------------|------------------------------------|-----------------|
| Serum Cystatin C | 0.97 ± 0.21 | 2.14 | 1.03 | 0.66 ± 0.14 | 1.4 ± 0.6 | 0.96 ± 0.29 |

Table 32 - Correlation of serum Cystatin C with other studies

Correlation of baseline characteristics with serum Cystatin C

1. Majority of the subjects with elevated Cystatin C were associated with a history of smoking and alcohol.
2. Subjects with elevated Cystatin C were also noted to have more complication. 23 out of 40 (57.50 %) had abnormal fundus, 22.5% (9) had neuropathy and 45% (18) had nephropathy on USG.
3. No significant difference was noted in the age and sex distribution between those with elevated and those with normal Cystatin C levels.
4. 55% (22) of the subjects with diabetes for 5-10 years had elevated Cystatin C levels. No significant difference was noted in the BMI between those with elevated and those with normal Cystatin C level.
5. 11 patients (27.50%) with normalalbuminuria had elevated Cystatin C
6. 22 patients (55%) with normal creatinine levels had elevated Cystatin C.
7. Cystatin C was elevated in 24 (60%) of subjects with GFR between 60-90 ml/min.

Glomerular filtration rate (GFR) (ml/min)

The GFR estimate was calculated from the Cockcroft-Gault formula and the subjects were divided into

three groups. The mean GFR in our study was 82.23 ± 22.3 . This correlated with the study by Yang YS et al study⁷².

51% of the subjects had GFR in the range of 60-90 ml/min, with majority among them in the normalalbuminuria group and with normal creatinine level. This was in contrast to the study by Yang YS et al⁷² where 40.4% were in 60-90 ml/min GFR range. Significantly GFR showed a negative correlation with albuminuria.

The methods used to determine GFR was the Cockcroft-Gault equation in study by Yang YS et al,⁷² Vishwanathan V et al⁷¹ and the present study. Piwowar A et al⁷³ study calculated GFR from the clearance of creatinine. Hoek et al⁶⁵ and Perkin BA et al⁷⁵ calculated GFR from the standard iothalamate clearance and creatinine clearance methods. The different methods used to measure GFR might explain the difference in the values of GFR obtained.

Comparison of markers

The comparison of markers was done after subclassifying the subjects based on the duration of diabetes and the different levels of GFR as already described.

Duration of diabetes

Most of the subjects of <5 years duration of diabetes were younger (31-50 yr) when compared with subjects with 5-15 years of diabetes (51-70). There was no significant difference in the sex distribution.

Majority of subjects in the <5 years group were noted to have normal serum creatinine levels (94%), with majority also showing normalalbuminuria (87.5%) and eGFR (53.57%). Cystatin C was elevated in only 18% the group.

A similar pattern was also noted in 5-10 year group with majority of subjects showing normal serum creatinine (71%), normalalbuminuria (50%) and elevated Cystatin C levels in 64.7% of the subjects in the group.

In the group with >10 yrs duration, 50% showed normal serum creatinine, 70% showed microalbuminuria and 90% had elevated Cystatin C

levels.

Serum Cystatin C was elevated even during the earlier stages of diabetic nephropathy when other markers were normal. The correlation of Cystatin C with other markers was statistically significant. ($r=0.593$ for ACR, $r=0.684$ for sCr).

GFR

The subjects were also subdivided into 3 groups based on the GFR estimated by the Cockcroft-Gault formula. An increasing trend was noted in serum creatinine values across the different stages of GFR. No significant difference was noted in BMI between those with elevated Cystatin C to those with normal Cystatin C levels.

Serum Cystatin C showed elevated levels with less GFR. There was a negative correlation between these two parameters which was statistically significant. ($r = -0.726$, $p = <0.01$).

Many previous studies have validated the use of Cystatin C as a marker in adult patients,⁵¹⁻⁵⁴ especially in comparison with serum creatinine,^{52-57,63,65-68} in different patient population.

Harmonien et al⁶⁷ studied 47 type 2 diabetic patients, showing that serum Cystatin C is more sensitive than sCr for the estimation of GFR when GFR is normal or slightly reduced. Mussap et al⁷⁶ also concluded that Cystatin C may be considered as an alternative to creatinine or the CG-CrI to discriminate normal from reduced GFR in type 2 diabetic patients. Both these studies kept Cr-51-EDTA clearance as the reference method for measuring GFR.

Tan et al⁷⁷ studied 29 type 1 diabetics and concluded that Cystatin C correlates with iohexol clearance better than creatinine.

After adjustment for age, Christensen et al⁷⁴ also demonstrated that serum Cystatin C is better to detect mild but not advanced diabetic nephropathy. Shimizu et al⁷⁸ showed a significant relationship between serum Cystatin C levels and the prognostic stages in patients with type 2 diabetic nephropathy.

On the other hand, Oddoze et al⁸⁰ demonstrated that serum Cystatin C is not more sensitive than

creatinine for detecting early renal impairment in patients with diabetes.

These studies differ from each other because of the use of different methods of GFR measurements, with different cut-offs levels of GFR (80, 68 or 60 ml/min), different methods of sCr and serum Cystatin C measurement.

Therefore, we studied the level of serum Cystatin C using the FDA approved method (immunonephelometry) in diabetic patients and analyzed the results in view of the markers frequently and routinely used in clinical practice, such as ACR, sCr and calculated CG-Cr.

The mean value of Cystatin C was increased in subjects with microalbuminuria; however it was also significantly increased even in the normoalbuminuric group. Serum Creatinine was increased only in the microalbuminuric group. There was positive correlation with ACR and creatinine levels ($r = .584$) as well as between ACR and Cystatin C level ($r = .593$).

There was a significant negative correlation between eGFR and ACR ($r = -0.684$) and eGFR and Cystatin C level ($r = -.726$).

The present study did not use isotopic or non-isotopic methods for the demonstration of GFR. However the superiority of Cystatin C and sCr with respect to GFR measurement has been confirmed by both superior correlation coefficients and greater ROC-plot AUC values in a meta-analysis.⁶⁸ In this large study, they used CCr as a GFR marker, because there was no gold standard for comparing the diagnostic accuracy of Cystatin C and sCr.

Further follow-up of renal function parameter changes for more accurate estimation of GFR is needed for type 2 diabetic patients. Recently, Perkins et al showed that serial measurements of serum Cystatin C could accurately detects trends in renal function in patients with normal or elevated GFR.⁷⁵ Thus, it is noteworthy to further study serum Cystatin C in CKD.

In conclusion, the correlation of serum Cystatin C with GFR was statistically significant. It was

elevated in many patient with <5 years duration of diabetes, with normal serum creatinine and normalalbuminuria. Further studies with standard clearance methods might be required to reassess this finding.

CONCLUSION

1. A higher percentage of the diabetic subjects were younger, were males and had diabetes for duration of < 5 years.
2. A higher percentage of subjects had normalalbuminuria.
3. More subjects in the proteinuric groups were associated with co-morbid condition and complication of diabetes and also had poorer glycemic control.
4. Most of the subjects who presented early had normal serum creatinine, normalalbuminuria with normal to slightly decreased GFR. Cystatin C levels were elevated in few of them
5. Serum Creatinine and Serum Cystatin C both correlated significantly with ACR and eGFR
6. However, most subjects with elevated Cystatin C had normal serum creatinine levels, normalalbuminuria and reduced GFR.

SUMMARY

The study was conducted in 100 diabetic subjects to study the correlation between serum creatinine, microalbuminuria and serum Cystatin C with diabetic nephropathy.

A majority of subjects with proteinuria were found to be associated with comorbid conditions, poorer glycemic control and more diabetic complications.

Serum creatinine showed a significant correlation with the albuminuria and the reduced GFR groups. Serum Cystatin C also showed a significant correlation with albuminuria and reduced GFR groups.

It was found that majority of subjects with lesser duration of diabetes had normal creatinine, normalalbuminuria, normal to reduced GFR but elevated Cystatin C levels in few of them.

Results of this study show that serum Cystatin C may be considered as an early marker, than microalbuminuria and serum creatinine, the commonly used marker for nephropathy, for declining renal function, in diabetic subjects. Further studies in larger population are needed to confirm this result.

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