Original Article

DOI: 10.7860/JCDR/2021/48031.15231

Microalbuminuria and Serum Cystatin C Correlation as Early Markers of Kidney Dysfunction in Patients with Type 1 Diabetes Mellitus

Biochemistry Section

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ABSTRACT

Introduction: Diabetes mellitus is the most common single cause of End Stage Renal Disease (ESRD). About 20-30% of patients with Type 1 Diabetes Mellitus (Type 1 DM) develop diabetic nephropathy as a serious complication which is the major cause of morbidity and mortality. Early identification of renal impairment is crucial to prevent the progression of nephropathy to a significant degree, because several interventions have greatest impact if initiated very early in the course of the disease.

Aim: To study the correlation of microalbuminuria and serum cystatin C for the early prediction of renal impairment in patients with Type 1 DM.

Materials and Methods: This was a cross-sectional study conducted from January 2016 to June 2016 on type 1 DM patients attending Diabetology Outpatient Department (OPD). According to the duration of diabetes, pateints were divided into two groups, Group I with <5 years and Group II with >5 years duration. The patients in each group were categorised as cases and controls, based on the Albumin Creatinine Ratio (ACR), serum urea, creatinine, cystatin C, glycated haemoglobin (HbA1c), and urine ACR levels were estimated. Student's unpaired t-test was used to compare the means between two independent groups. Pearson correlation coefficient was used to estimate the degree of correlation between two quantitative variables.

Results: Seventy-two patients with type 1 DM were included in the study. Cystatin C levels in cases (0.89±0.35) when compared to controls (0.67±0.26) in more than five years duration of type 1 DM was found to be statistically significant ($p<0.01$). Positive correlation between cystatin C and ACR ($r=0.4$, $p<0.05$) was found to be statistically significant in more than five years duration. It indicates that serum cystatin C levels increases as the values of ACR increases. There was no significant difference in cystatin C levels in patients with less than five years duration of type 1 DM.

Conclusion: Serum cystatin C may be considered as an early predictor of renal impairment in type 1 DM patients with more than five years duration. However, in this study cystatin C carries no significance in less than five years duration of type 1 DM patients.

Keywords: Diabetic nephropathy, End stage renal disease, Urine albumin creatinine ratio, Urine albumin excretion

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterised by hyperglycamia due to defect in insulin secretion, action or both [1]. The prevalence is rising for the past two decades. Among 8.3% of world's adult population with diabetes, its prevalence varies between 11.6% to 30.9% and in India it is 9% [2]. The existence also surges with age about 0.2% for <20 years, 11.3% for >20 years and 26.9% for >65 years of age. Male and female prevalence is similar about 11.8% and 10.8%, respectively. DM is the primary cause of End Stage Renal Disease (ESRD). It is the fifth important cause of death (6.8%) worldwide [3].

The American Diabetes Association (2011) classified DM into type 1 DM, type 2 DM and other specific types. Type 1 DM is due to autoimmune destruction of beta cells of pancreas, leading to defect in insulin production and secretion. Though it can occur at any age, most type 1 DM cases develop before 30 years [4]. It starts in children at 4 years and reaches the peak at 11 to 13 years of age [5]. Type 1 DM has 25-30% risk of developing diabetic nephropathy [6] which is a clinical syndrome characterised by persistent albuminuria (>300 mg/24 hrs or >300 mg/g of creatinine), decline in Glomerular Filtration Rate (GFR) (2-20 mL/min/year), elevated arterial blood pressure and increased cardiovascular morbidity and mortality [7]. It is a serious complication and becoming the most important cause for ESRD, leading to death [8]. In due course, 40% of patients with type 1 DM develop (ESRD) [9]. Timely detection of kidney dysfunction is crucial to prevent the progression of ESRD in these patients [10].

Microalbuminuria (moderately increased albuminuria) is a clinically important marker of declining renal function in diabetic patients. Measurement of ACR is used to find out the Urine Albumin Excretion (UAE) [11]. Although UAE is the cornerstone for detecting early kidney dysfunction, few patients do have normal UAE despite decline in renal function. Even diagnosis of microalbuminuria may be too late for preventing the development of nephropathy. For this reason, other markers can be utilised, which may be beneficial for the prediction of early renal impairment in type 1 diabetic patients.

At present, renal function is assessed by using serum creatinine values. But it may not be sufficient to detect early renal dysfunction as its level remains in the normal range until 50% of renal function is lost. Also, serum creatinine values are altered by some of the individual variations like age, gender, muscle mass and nutritional status.

The protein cystatin C is a low molecular weight (12.8 kDa) cysteine protease inhibitor, which is freely filtered by the glomerulus, but not secreted by the tubules and it is unaffected by muscle mass, diet and gender is completely eliminated from the circulation. For this reason, cystatin C measurement is more sensitive and specific than creatinine for renal function assessment. Cystatin C concentration is increased earlier than creatinine as GFR declines below 80 mL/min/1.73 m^2. The sensitivity and specificity of Cystatin C is about 70-87% and 96%, respectively, which is much higher compared to creatinine. This makes cystatin C as a better indicator for impaired renal function.

The present study was aimed to evaluate the likelihood of serum cystatin C as an early marker of kidney dysfunction and to determine

the association between microalbuminuria and cystatin C in type 1 DM patients.

MATERIALS AND METHODS

This cross-sectional study was conducted from January 2016 to June 2016 on patients who attended the Diabetology OPD of the Institute of Diabetology at Government Stanley Medical College and Hospital, Chennai. Institutional Ethics Committee (IEC) permission was obtained before starting the study (IEC, Stanley Medical College, Chennai. Dated on 13.01.2016). Blood samples were collected from 72 patients of type 1 DM. The following inclusion and exclusion criteria were followed:

Inclusion criteria: Patients diagnosed as type 1 DM.

Exclusion criteria: Type 1 DM patients associated with hypertension, urinary tract infection (UTI), congestive heart failure and thyroid dysfunction.

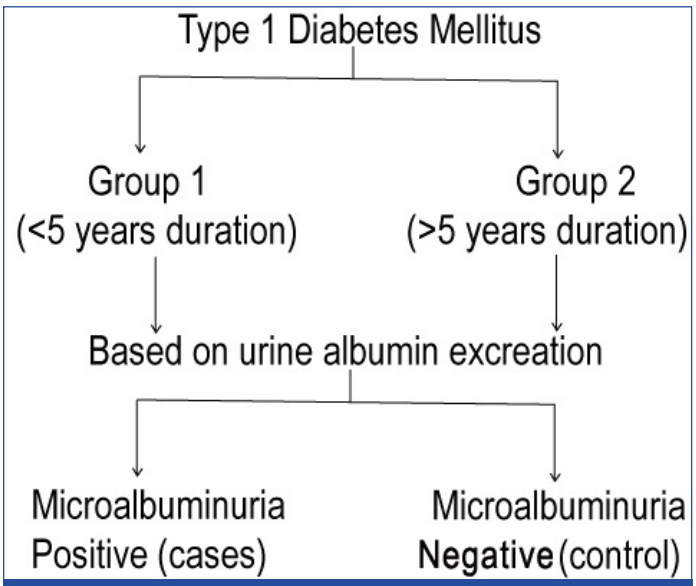
Study Procedure

Patients who met the inclusion criteria, after getting informed consent, were divided into two groups.

Group I: Patients with less than 5 years duration of type1 DM.

Group II: Patients with more than 5 years duration of type 1 DM.

In each group microalbuminuria positive pateints were taken as cases and microalbuminuria negative pateints were taken as controls [Table/Fig-1]. Serum cystatin C and creatinine levels were estimated in both cases and controls then they were correlated with urine albumin levels.



[Table/Fig-1]: Flowchart of distribution of subjects.

Sample Collection and Preparation

After obtaining informed consent from the patients, blood samples were collected randomly under strict aseptic precautions in plain red capped vacutainer tubes. Ethylene Diamine Tetraacetic Acid (EDTA) tubes were also used to collect samples for HbA1c estimation. Samples were centrifuged at 2000-2500 rpm for 10 minutes. Sera were separated immediately and stored at -20ºC in deep freezer. Cystatin C levels can remain stable for upto i.e., three months at -20ºC.

ACR was estimated by collecting spot urine sample in a clean container. Once the sample became positive for microalbumin by dipstick method, a second sample was analysed within one month duration to confirm microalbuminuria by latex turbidimetry, which is a method used for quantitative measurement of microalbumin in urine.

The study participants were examined and microalbumin, creatinine, ACR were estimated in urine samples. Urea, creatinine, cystatin C, HbA1c were estimated in serum samples. Urine microalbumin was estimated by latex turbidimetry, serum urea was estimated by urease coupled with glutamate dehydrogenase method, serum/urine creatinine was estimated by modified Jaffe's method, serum cystatin C was estimated by colloidal gold-enhanced turbidimetric immunoassay using Beckman Coulter AU 480 autoanalyser. HbA1c was estimated by ion exchange high performance liquid chromatography using Bio-Rad D10 analyser.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) software version 16. Student's unpaired t-test was used to compare the means between two independent groups (cases and controls of Group I and Group II). Pearson correlation coefficient was used to find out the correlation between microalbuminuria and cystatin C. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Seventy-two patients with type 1 DM were included in the study. They were divided into two groups according to the duration of diabetes. Among them 17 were categorised into group I and 55 were categorised into Group II [Table/Fig-2] shows the distribution of subjects in both the groups. The number of subjects categorised into Group II is more than that of Group I.

	Group I (n=17)		Group II (n=55)	
Groups	n	%	n	%
Cases	10	58.8	20	36.4
Controls	7	41.2	35	63.6
Total	17	100	55	100

[Table/Fig-2]: Distribution of Subjects between two groups (N=72).

[Table/Fig-3] shows the different biochemical parameters of the study population. Among the parameters compared between group I and II, creatinine and cystatin C showed statistically significant difference between the two groups with p-value of <0.05. [Table/Fig-4] shows the comparison of biochemical parameters between cases and controls in Group I with statistically significant difference in ACR between cases (96.99±51.71) and controls (19.79±7.28) with p-value <0.002. The other parameters of urea, creatinine, cystatin C and HbA1c were not statistically significant. [Table/Fig-5] shows the same parameters in Group II, which revealed statistically significant difference (p<0.05) in ACR, cystatin C and HbA1c between cases and controls.

Parameters	Group I (Mean±SD)	Group II (Mean±SD)	p-value
ACR	65.2±55.3	51.5±57.5	0.174
Urea	19.64±6.15	20.63±6.51	0.580
Creatinine	0.84±0.16	0.95±0.19	0.027
Cystatin C	0.67±0.15	0.81±0.30	0.015
HbA1c	8.98±1.92	9.13±1.94	0.783

[Table/Fig-3]: Biochemical parameters of the study population.
(p<0.05 -Statistically significant); ACR: Albumin creatinine ratio

Parameters	Cases (Mean±SD)	Controls (Mean±SD)	p-value
ACR	96.99±51.71	19.79±7.28	0.002
Urea	19.70±5.96	19.57±6.90	0.967
Creatinine	0.81±0.17	0.90±0.14	0.268
Cystatin C	0.68±0.18	0.66±0.13	0.804
HbA1c	9.64±2.16	8.04±1.03	0.550

[Table/Fig-4]: Comparison of biochemical parameters between cases and controls in group I (<5 years).
(p<0.05- Statistically significant); ACR: Albumin creatinine ratio

[Table/Fig-6] indicates the Pearson's correlation between cystatin C, creatinine, HbA1c with ACR in Group I which showed r=0.02, r=0.01, r=0.03 respectively, which is not statistically significant. [Table/Fig-7] shows significant positive correlation between cystatin C and ACR (r=0.4, p<0.05), creatinine and ACR (r=0.2, p<0.05), HbA1c and ACR (r=0.1, p<0.05) in Group II.

Parameters	Cases (Mean±SD)	Controls (Mean±SD)	p-value
ACR	109.60±60.88	18.34±8.11	<0.001
Urea	20.15±6.25	20.91±6.73	0.681
Creatinine	0.96±0.22	0.95±0.18	0.855
Cystatin C	0.89±0.35	0.67±0.26	0.010
HbA1c	10.18±1.91	8.52±1.71	<0.001

[Table/Fig-5]: Comparison of biochemical parameters between cases and controls in group II (>5 years).
p<0.05- Statistically significant; ACR: Albumin creatinine ratio

Parameters	Pearson's correlation significance Group I
Cystatin C vs ACR	r=0.02
Creatinine vs ACR	r=0.01
HbA1c vs ACR	r=0.03

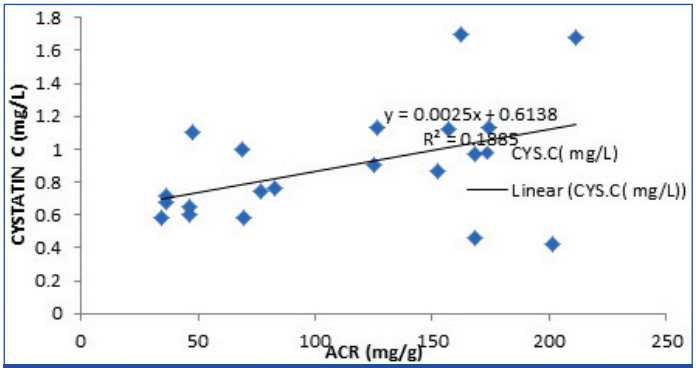
[Table/Fig-6]: Pearson's correlation between cystatin C, creatinine, HbA1c with ACR in group I (<5 years) patients.
ACR: Albumin creatinine ratio

Parameters	Pearson's correlation coefficient Group II
Cystatin C vs ACR	r= 0.4
Creatinine vs ACR	r= 0.2
HbA1c vs ACR	r= 0.1

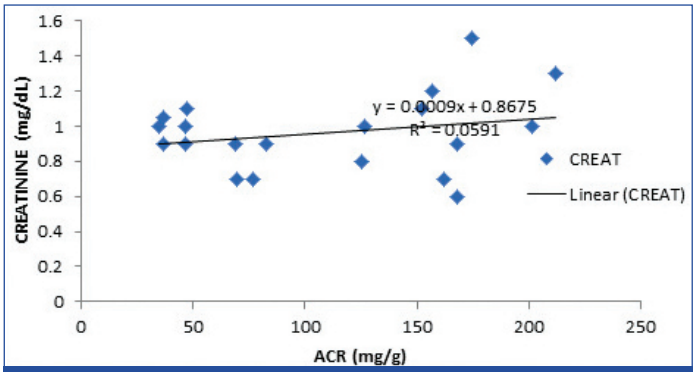
[Table/Fig-7]: Pearson's correlation between cystatin C, creatinine, HbA1c with ACR in group II (>5 years) patients.

[Table/Fig-8] shows a linear regression analysis between cystatin C vs ACR and [Table/Fig-9] shows a linear regression analysis between ACR vs creatinine, which revealed positive correlation for both. However, changes in ACR can be better explained by changes in cystatin C values (R^2=0.1885) compared to that of creatinine (R^2=0.0591) justifying cystatin C role as a better predictor of changes in microalbuminuria than creatinine.

[Table/Fig-10] shows student t-test between different analytes among Group II, which showed statistically significant difference (p<0.05) between cystatin C vs ACR, creatinine vs ACR, HbA1c vs ACR, suggesting that these variables are independent of each other.



[Table/Fig-8]: Regression analysis between ACR and cystatin C in group II.
ACR values have a positive correlation with cystatin C as indicated by the upward slope of linear regression analysis and the r value is equal to 0.4



[Table/Fig-9]: Regression analysis between ACR and creatinine in group II.
ACR values have a positive correlation with creatinine as indicated by the upward slope of linear regression analysis and the r value is equal to 0.2

Analytes	t-value	Significance
Cystatin C vs ACR	7.98	<0.001
Creatinine vs ACR	7.65	<0.001
HbA1c vs ACR	7.29	<0.001

[Table/Fig-10]: Unpaired 't' test between different analytes among group II cases of type 1 diabetes mellitus.
p-value <0.05 considered significant

DISCUSSION

Diabetic patients are more prone to develop kidney disease. The peak onset of diabetic nephropathy occurs between 10-15 years of duration of diabetes [12]. There is an unmet need for high sensitive biomarkers to detect diabetic nephropathy. As there is no adequate diagnostic support available for the early detection of nephropathy, the chances are very high for early nephropathy and microalbuminuria to progress to ESRD [13].

Microalbuminuria, elevated serum creatinine and reduced GFR were commonly used measures for the detection of diabetic nephropathy [14]. But this microalbuminuria may present in non diabetic renal disease, menstrual contamination, uncontrolled hypertension, Urinary Tract Infection (UTI) and strenuous exercise. Also, the decline in renal function in diabetes is not always accompanied by an increased ACR [15]. Early structural damage in both glomerular and tubular structures may be present in patients with type 1 DM with normal UAE.

To overcome this, eGFR which is calculated from creatinine value was used to evaluate the renal function. Since creatinine levels were affected by age, sex, muscle mass and nutritional status; cystatin C which is not affected by individual variation was used for assessment of renal impairment. So, the present study was conducted to identify the correlation of cystatin C with ACR for the detection of early renal impairment in type 1 DM. Cystatin C is more effective in early detection even before the development of microalbuminuria, as its concentration started to increase as GFR fell below 80 mL/min/1.73 m^2 compared with about 40 mL/min/1.73 m^2 for creatinine [16]. So the use of cystatin C may further unravel the pathophysiological changes in type I DM.

The present study revealed elevation of cystatin C, creatinine, ACR, and HbA1c levels among cases of more than five years duration of diabetes when compared with less than five years duration. These results are along similar lines with the conclusion of Brijesh M, who indicated that the prevalence of diabetic nephropathy was linked to the duration of diabetes and glycaemic control [17]. The progression of the disease is directly proportional to the duration of diabetes and poor glycaemic control.

ACR revealed statistically significant difference (p<0.05) between cases and controls in Group I, which is similar to study done by Alaaeldin M and Aly A, which states that screening for microalbuminuria may be estimated one year after diagnosis of type 1 DM. The EURO DIAB IDDM demonstrated that the prevalence of microalbuminuria may reach 18% before 5 years in Type 1 DM [18] and Group II which is consistent with Saurav P who make it evident that the level of microalbumin in urine increases with increasing duration of diabetes [19]. In this study, authors found statistically significant (p<0.05) elevation of cystatin C in cases when compared to controls in Group II with more than five years duration which is consistent with Asssal HS and Tawfeek S [20] states that cystatin C is elevated in Group II but creatinine levels are not significantly increased (p>0.05). It assumes that serum cystatin C is an early marker of diabetic nephropathy in Contrast to Serum Creatinine.

The current study revealed a significant positive correlation between cystatin C and ACR (r=0.4, p<0.05) in Group II with more than five years duration which is concordant with Jeon YK et al., [21]. This shows that serum cystatin C levels increase as ACR increases, which means that more severe the renal impairment, the higher the values of cystatin C.

The HbA1c levels were increased in cases compared to the controls in Group II with p-value <0.05. This finding is similar with Straton IM and Adler AI [22] who established that the microvascular complications were associated with poor glycaemic control. This confirms that the occurrence of diabetic nephropathy increases parallel with the progression of the disease and poor glycaemic control.

Even though our study revealed positive correlation between creatinine with cystatin C (r=0.2, p<0.05) in Group II, the patients in this group had normal creatinine level with microalbuminuria, elevated cystatin C, which is in accordance with Christensson AG suggested that cystatin C performed better than creatinine to detect mild diabetic nephropathy as defined by GFR <80 mL/min/1.73 m^2 [23]. However the efficacy of cystatin C for estimating GFR has not been adequately established in children with diabetes.

This study also revealed the positive correlation between HbA1c and ACR in Group II (r=0.1, p<0.05) signifying that kidney dysfunction in type 1 DM rises with poor glycaemic control. Kapstein et al., found parallel outcome that ACR and HbA1c have positive correlation in diabetic patients [24].

Limitation(s)

Microalbuminuria and cystatin C correlation is not obtained in less than five years duration of Type 1 DM, may be because of small number of patients enrolled in this group.

CONCLUSION(S)

Microalbuminuria is moderately correlated with cystatin C, suggesting that cystatin C can be used as an early marker of kidney impairment in type 1 DM with more than five years duration, which will allow timely intervention and management of diabetic nephropathy.

Acknowledgement

We sincerely thank our Assistant Professors, Associate Professors and Professor and HOD, Department of Biochemistry, Professor and HOD Department of Nephrology and Associate Professor of Diabetology, Government Stanley Medical College and Hospital for their immense support and valuable suggestions. Finally, we would show gratitude to all the participants who united with us during the course of our work.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Dec 07, 2021
- Manual Googling: Mar 15, 2021
- iThenticate Software: Jun 08, 2021 (20%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Dec 04, 2020**
Date of Peer Review: **Feb 15, 2021**
Date of Acceptance: **Apr 09, 2021**
Date of Publishing: **Aug 01, 2021**