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A comparative study of serum cystatin C levels in Type 2 diabetes mellitus with and without microalbuminuria

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ABSTRACT

Background: Serum Cystatin C is elevated in diabetic patients even before the appearance of microalbuminuria and hence, it can be used as an early marker for detecting diabetic nephropathy. **Aims and Objectives:** The aim of the study was to compare serum Cystatin C levels in Type 2 diabetes mellitus (DM) patients with microalbuminuria and without microalbuminuria. **Materials and Methods:** A hospital-based and cross-sectional study conducted among 40 patients with Type 2 diabetic mellitus attending the Endocrinology OPD Medical College, Thiruvananthapuram. Informed consent was obtained. Biochemical parameters like blood glucose was estimated by hexokinase method and serum creatinine by modified Jaffe's method using fully automated analyzer. HbA1c was determined by turbidimetric immunoinhibition method. Serum Cystatin C and microalbumin was evaluated by ELISA method. Statistical analysis of data was performed using SPSS windows version 26. Statistical tests like unpaired t-test were done to compare the study subjects and Pearson correlation was done to know the correlation between variables and $P<0.05$ was considered as significant. **Results:** In this study, all the study group had fasting blood level above 126 mg/dL with HbA1c value >6.5%. Serum creatinine value <1.4 mg/dL is considered normal. In this study group subjects, 34 (85%) had normal level of serum creatinine, while 6 (15%) 6 patients had creatinine level more than 1.4 mg/dL. Mean ± SD for serum cystatin C levels in Type 2 DM patients with microalbuminuria and without microalbuminuria was 22.7 ± 4.9 and 4.79 ± 3.55, respectively. The serum cystatin C levels were significantly ($P<0.01$) higher in the microalbuminuria group than in the normoalbuminuric patients. In the present study, there was highly significant positive correlation between serum cystatin C and microalbumin ($r=0.93$, $P<0.001$. In the present study, there was highly significant positive correlation between serum cystatin C and fasting blood sugar, serum creatinine, and glycated hemoglobin in patients with microalbuminuria) than without microalbuminuria. **Conclusion:** Serum cystatin C levels were found to be increased in patients with Type 2 DM with microalbuminuria and can be considered as a valuable early marker of renal damage.

Key words: Diabetic nephropathy; Microalbumin; Serum creatinine; Serum cystatin C; Type 2 diabetes

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INTRODUCTION

One in 11 persons worldwide have diabetes mellitus (DM), of which 90% have Type 2 DM.[1] Serum creatinine, estimated glomerular filtration rate (eGFR), blood urea, and urine albumin are typical indicators for the diagnosis and progression of diabetic nephropathy (DN).[2-11] Serum creatinine and eGFR are commonly used to evaluate renal function. However, it is noteworthy that a number of non-renal variables, including age, weight, nutritional status, race, and gender, can affect serum creatinine levels.[12]

In the creatinine blind area, where the renal function is known to be somewhat impaired (40–70 mL/

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min/1.73 m^2), it is also known to have low sensitivity.[13] About 46% of older adults with chronic renal illness in India have DN, which is linked to higher cardiovascular mortality and morbidity. In Indian diabetic individuals, the prevalence of DN has dramatically increased and is now the only known cause of end-stage kidney disease. In the USA, Europe, Japan, and other Asian nations, DN is the most well-known cause of end-stage renal disease, accounting for 25–45% of all patients enrolled in ESRD programs worldwide. With an eGFR of <60 mL/min/1.73 m^2 or a urine albumin/creatinine ratio of >30 mg/g for more than 2 years, DN is said to be chronic kidney disease caused by DM.[9,14]

Regular traditional examination of DN uses microalbuminuria. With some restrictions, higher serum creatinine, microalbuminuria, and reduced creatinine clearance aid in the diagnosis of DN. As it seems to be less influenced by elements known to skew creatinine concentrations, such as muscle mass and protein intake, cystatin C is being explored as a possible replacement for serum creatinine.[15]

Aim

The aim of the study was to compare serum Cystatin C levels in Type 2 diabetes mellitus (DM) patients with microalbuminuria and without microalbuminuria.

Primary objectives

To compare serum cystatin C levels in type 2 diabetes mellitus patients with microalbuminuria and without microalbuminuria

Secondary objectives

1. To correlate serum cystatin C levels with urine in type 2 diabetes mellitus patients with microalbuminuria
2. To correlate serum cystatin C levels with glycated hemoglobin in levels in type 2 diabetes mellitus.

MATERIALS AND METHODS

This hospital-based and cross-sectional study involved 40 Type 2 diabetic patients who attended Endocrinology OPD Medical College in Thiruvananthapuram from August 2018 to July 2019. This study got approval from Ethics committee of the institution under the letter number IEC.NO.13/09/2017/MCT. Out of the 40 diabetic patients, 20 patients were with microalbuminuria and the other 20 patients without microalbuminuria.

Inclusion criteria

Inclusion criteria were as follows: Type 2 DM adult patients of both sexes of age group 50–80 years.

Exclusion criteria

Exclusion criteria were as follows: Patients with history of renal disease, liver disease, infections, and thyroid disease or other endocrine diseases were excluded from this study.

The study population included was given a pre-made questionnaire and informed consent was obtained. Biochemical parameters such as blood glucose (hexokinase method) and serum creatinine (modified Jaffe's method) were estimated in automated analyzers. Turbidimetric immunoinhibition was used to measure HbA1c. Microalbumin and serum Cystatin C were measured by ELISA. SPSS Windows version 26 was used for the statistical analysis of the data. Data were summarized using mean and standard deviation. To compare the study subjects, the unpaired t-test was conducted. Pearson correlation was done to study the correlation between the variables and $P<0.05$ was considered significant.

RESULTS

In the present study, 40 Type 2 diabetic individuals of both sexes with ages between 50 and 80 were included. In the present study, maximum amount of subject fall in the age group below 60 years, about 55%. Only 14 (35%) subjects

Table 1: Sociodemographic variable in the study group

Age	Count	Percent
50–59	22	55.0
60–69	14	35.0
70–79	4	10.0
Mean±SD	59.6±6.3	

Table 2: Sex distribution in the study group

Sex	Count	Percent
Male	19	47.5
Female	21	52.5

Table 3: Biochemical parameters

Fasting blood sugar			
Blood level	**Frequency**	**Percent**	**Total**
<100%	0	0	100.0
100–125%	0	0	
>126%	40	100.0	
	40		
HbA1C			
<5.5%	0	0	100.0
5.6–6.4	0	0	
>6.5	40	100.0	
Serum creatinine			
<30 µg/mL	34	85	100.0
>30 µg/mL	6	15	

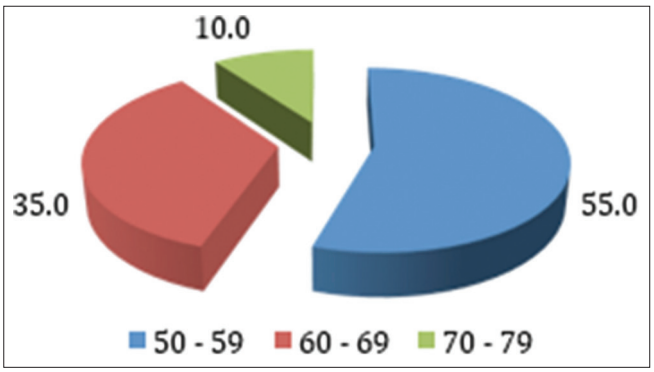

Figure 1: Percentage distribution of the sample according to age

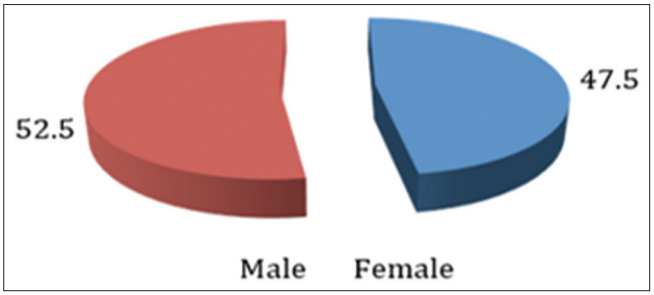

Figure 2: Percentage distribution of the sample according to sex

belong to age group above 60–69 years and 4 (10%) subjects belong to age group above 70–79 years (Table 1 and Figure 1).

About 52.5% of the study subjects were females, the rest being males (Table 2 and Figure 2).

In this study, all the participants (100%) had fasting blood sugar (FBS)>126% and HbA1C value >6.5, whereas, 6 (15) out of 40 participants has serum creatinine >30 μg/mL (Table 3).

Patients with microalbuminuria had a mean (mean+SD) serum Cystatin C value of 22.7±4.79, while those without the condition had a value of 4.9±3.55. Serum Cystatin C exhibited a statistically significant positive connection (r=0.93, P=0.001) with urine microalbumin, serum creatinine (r=0.85, P=0.001), FBS (r=0.993, P=0.0001), and HbA1c (r=0.964, P=0.001) (Tables 4-6 and Figures 3 and 4).

In the present study, there was highly significant positive correlation between serum cystatin C and microalbumin (r=0.93, P<0.001) (Figure 5).

In the present study, there was highly significant positive correlation between serum cystatin C and FBS. It was higher in microalbuminuria than non-albuminuria (Figure 6).

In the present study, there was highly significant positive correlation between serum cystatin C and glycated hemoglobin (Figure 7).

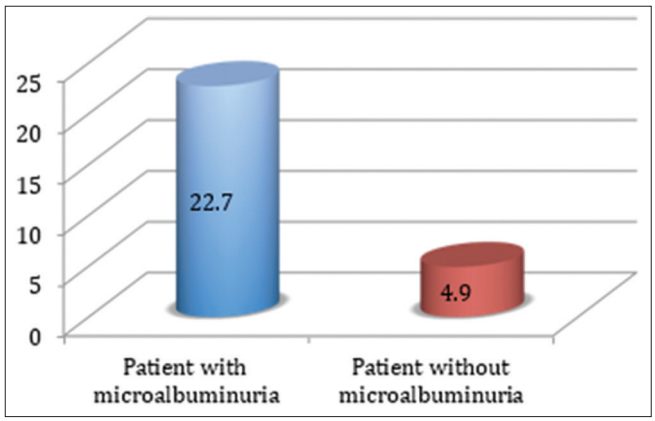

Figure 3: Serum cystatin C levels in Type 2 diabetes mellitus patients with microalbuminuria and without microalbuminuria

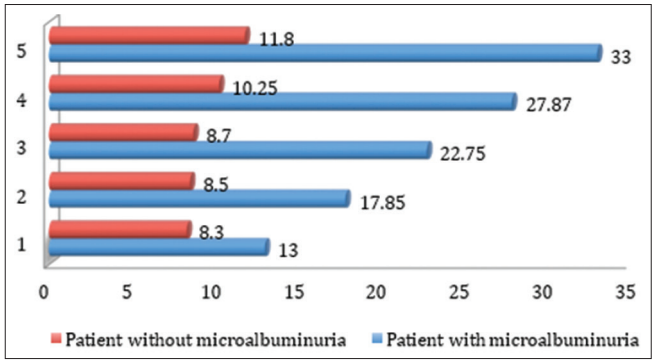

Figure 4: Bar chart for serum cystatin C level based on group

Table 4: Mean±SD in patients with/without microalbuminuria

Statistical parameter	Patients with microalbuminuria (20)	Patients without microalbuminuria (20)
Mean	22.7	4.9
SD	4.79	3.55
Q1	17.85	8.5
Median	22.75	8.7
Q3	27.87	10.25
Minimum	13	8.3
Maximum	33	11.8

SD: Standard deviation

DISCUSSION

The most common cause of end-stage renal disease is DN and hyperglycemia is a major factor in the onset of DN. Renal damage is brought on by increased hyperfiltration due to hyperglycemia. According to broad consensus, hyperglycemia is the primary catalyst for renal impairment because it activates other metabolic pathways and causes an increase in oxidative stress. Microalbuminuria, which is also known as DN and is associated with severe glomerular damage, is thought to be an early warning sign of renal impairment in people with Type 2 DM.

Table 5: Serum cystatin C levels in Type 2 diabetes mellitus patients with microalbuminuria and without microalbuminuria

Group	Mean	SD	n	t	P-value
Patients with microalbuminuria	22.7	4.79	20	13	P<0.01
Patients without microalbuminuria	4.9	3.55	20		

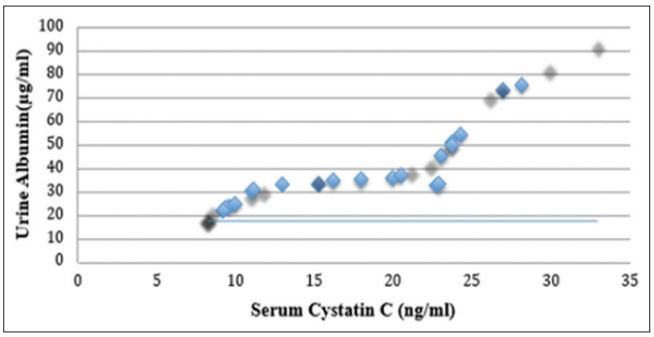

Figure 5: Correlation of serum cystatin C with microalbumin r=0.93, P=0.001

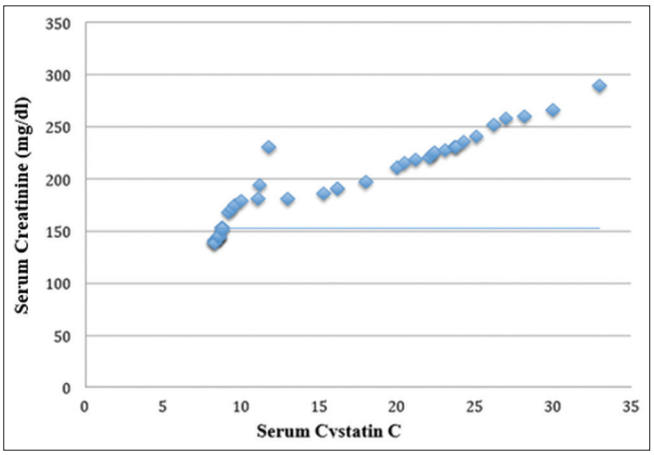

Figure 6: Scatter diagram for FBS and serum cystatin C level

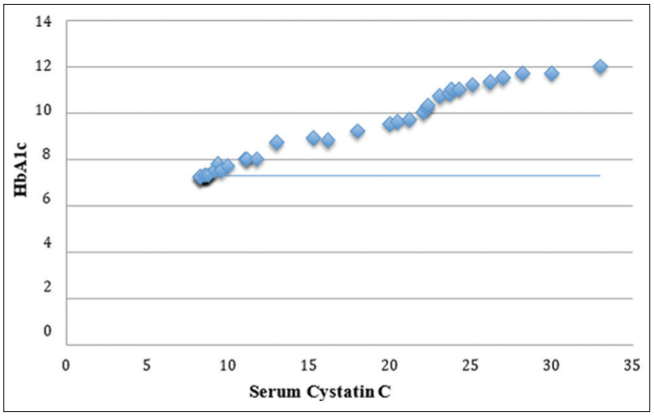

Figure 7: Scatter diagram for HbA1c and serum cystatin C level

According to recent investigations, normal albuminuria may reveal structural renal damage in both the tubular and glomerular portions, which is not always reflected by microalbuminuria.

The morbidity and mortality linked to DM are caused by secondary pathophysiological changes brought on by the metabolic dysregulations associated with the disease in numerous organ systems, which lead to a number of consequences. In Type 2 DM patients, DN, a microvascular consequence, is the main cause of morbidity and mortality. Tubulointerstitial injury may be a significant part in the pathogenesis of DN, even though glomerular dysfunction is regarded to be a major contributor in the onset and continued advancement of DN. Early recognition of these alterations has the potential to slow or stop the progression of the complications.

In this hospital-based and cross-sectional investigation, serum cystatin C levels were compared in Type 2 DM patients with and without microalbuminuria, as well as their levels in relation to serum creatinine and glycated hemoglobin.

In this study, 40 Type 2 diabetics with a diagnosis of diabetes for more than 5 years who were between the ages of 50 and 80 were included in the study. In the study group, 45% of participants were older than 60 and 55% were under 60. The individuals in the present study have an average age of 59.6±6.3 years.

About 52.5% of the participants in this study were females, whereas 47.5% were men. Similar findings were observed in the study by Pavkov et al., and Al-Saedy et al.[15,16]

In the study group, the mean FBS was 190.4±41.5 mg/dL. Between the study groups, there was a statistically significant difference in the blood glucose levels (P=0.0001). In comparison to non-albuminuria, it was significantly higher in microalbuminuria. All the subjects in the study population had HbA1c >6.5% indicating poor glycemic control with mean value 8.9±1.6%.

Glycated hemoglobin levels showed statistically significant difference (P=0.001) among studied groups. It was significantly higher in microalbuminuria than non-albuminuria. In the studies conducted by Al-Saedy et al., and Pavkov et al., the mean value of HbA1c was >6.5%.[16,17]

Of the study participants, 50% (20) had normal albumiuria and 50% (20) had microalbuminuria. There was significant change in albumin level in the study population. Mean microalbumin level was 36.35±19.14 µg/mL (Table 5).

Table 6: Correlation of serum cystatin C levels with fasting blood sugar in Type 2 diabetes mellitus patients with and without microalbuminuria

Group	r	P-value
Patients with microalbuminuria	0.993**	0.0001
Patients without microalbuminuria	0.952	0.0001

**Significant at 0.0001 level

Table 7: Correlation of serum cystatin C levels with Serum Creatinine in Type 2 diabetes mellitus patients with and without microalbuminuria

Group	r	P-value
Patients with microalbuminuria	0.85	0.0001
Patients without microalbuminuria	−0.16	0.488

Correlation of serum cystatin C levels with fasting blood sugar in Type 2 diabetes mellitus patients with and without microalbuminuria with the 'r' value of 0.993 and 0.962 respectively with a 'p' value of 0.0001 (Table 6).

Correlation of serum cystatin C levels with Serum Creatinine in Type 2 diabetes mellitus patients with and without microalbuminuria was 0.85 and - 0.16 with a 'p' value of 0.0001 and 0.488 respectively (Table 7).

In the study population, 6% of patients showed serum creatinine value above normal. About 85% showed normal creatinine level. The mean value of serum creatinine was 1.29±1.28 mg/dL. Serum creatinine levels were significantly higher in microalbuminuria than non-albuminuria (P<0.001).

Serum cystatin C levels averaged 15.98+7.62 ng/mL. Twenty of the participants (patients with microalbuminuria) had high blood cystatin C levels (22.7±4.79 ng/mL), whereas 20 of the subjects (patients without microalbuminuria) had serum cystatin C levels of 4.9±3.55 ng/mL (Table 5). Among the groups under study, serum cystatin C levels considerably rose (P=0.0001). When compared to diabetic individuals without albuminuria, diabetic patients with microalbuminuria had a significantly higher level of serum cystatin C in this study. This result is consistent with research by Pavkov et al., and Al-Saedy et al.[16,17] In these patients, there was a highly significant positive association between serum cystatin C and microalbumin (r−0.93, P=0.001). These findings also support Bassiouny et al., study, which found a substantial positive correlation with urine albumin excretion and serum Cystatin C.[18]

In the present study, patients with microalbuminuria (r=−0.99, P=−0.0001) and those without microalbuminuria (r=−0.952, P−0.001) showed a strong association between serum cystatin C and FBS similar to study by Al-Saedy et al.[16]

There was a significant positive correlation between HbA1C and serum Cystatin C in patients with microalbuminuria (r=−0.964, P=−0.001) and patients without microalbuminuria (r=−0.953, P=−0.001) which is consistent with study by Pavkov et al., and Al-Saedy et al.[16,17]

In this study, 14 patients in the study group had normal creatinine levels but had elevated serum cystatin C values and six patients had elevated levels of both serum creatinine and serum cystatin C. Significant positive correlation was seen between serum cystatin C with microalbuminuria and serum creatinine (r=–85, P<0.001). Suzuki et al., found a strong association between serum cystatin C and serum creatinine.[19]

An early indicator of renal impairment in people without microalbuminuria is therefore necessary. Even in patients without albuminuria, serum Cystatin C is an early indicator of DN, and it positively correlates with microalbuminuria as renal impairment worsens.

Limitations of the study

1. The study sample was small and the study design was cross sectional.
2. The study was hospital based and it may not fully represent the diabetic population of the state.
3. The serum cystatin c was measured only in cases. No controls were taken in this study.
4. Only patient with age above 50 and below 80 were taken as cases.
5. No methods were used to relate the serum cystatin c levels and the degree of tubular damage.
6. Large, multicentre prospective studies are still needed to confirm its clinical utility as serum marker in early diabetic nephropathy for everyday practice.

CONCLUSION

When compared to diabetic patients without albuminuria, diabetic patients with microalbuminuria have a higher level of serum cystatin C, which is highly significant in the current study. Poor glycemic control in these patients were indicated by their high mean FBS and HbA1c values. Serum creatinine, FBS, glycated hemoglobin, and urine microalbumin all showed significantly positive correlation with serum cystatin C in the study group.

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Authors Contribution:
BSL- Definition of intellectual content and design of study, coordination clinical protocol, implementation of study protocol, literature survey, data collection, data analysis, and prepared tables; **ACJ-** Concept, statistical analysis and interpretation, manuscript preparation, editing, revision, and submission of article.

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