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Study of Cystatin C as Early Biomarker of Nephropathy in Patients with Type 2 DM and Risk Stratification in Tarnaka Hospital of Hyderabad City in India

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Abstract: This study was done to evaluate clinical usefulness of cystatin C levels of serum and urine in predicting renal impairment in normoalbuminuric patients with type 2 diabetes and to evaluate the association between albuminuria and serum/urine cystatin C. Type 2 diabetic patients (n = 200) with normoalbuminuria (n = 45), microalbuminuria (n = 83) and macroalbuminuria (n = 42) were enrolled. Creatinine, urinary albumin levels, serum/urine cystatin C and estimated glomerular filtration rate (eGFR by MDRD (Modification of Diet in Renal Disease) and CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration] equations)) were determined. The cystatin C levels of serum and urine increased with increasing degree of albuminuria, reaching higher levels in macroalbuminuric patients ($p < 0.001$). In multiple regression analysis, C-reactive protein (CRP), sex, albumin-creatinine ratio (ACR) and eGFR affected serum cystatin C. Urine cystatin C was affected by triglyceride, age, eGFR and ACR. In multivariate logistic analysis, cystatin C levels of serum and urine were identified as independent factors associated with eGFR < 60 mL/min/1.73 m^2 estimated by MDRD equation in patients with normoalbuminuria. On the other hand, eGFR < 60 mL/min/1.73 m^2 estimated by CKD-EPI equation was independently associated with low level of high-density lipoprotein in normoalbuminuric patients. The cystatin C levels of serum and urine could be useful markers for renal dysfunction in type 2 diabetic patients with normoalbuminuria.

Key words: Cystatin C, diabetic nephropathies, albuminuria.

1. Introduction

The number of people with diabetes is increasing due to population growth, aging, urbanization and the increasing prevalence of obesity and physical inactivity. According to the World Health Organization (WHO), the prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030 [1]. Estimation of the prevalence of earlier stages of chronic kidney disease (CKD) in the US population and ascertainment of trends over time is central to disease management and prevention planning, particularly given the increased prevalence of obesity and diabetes [2]. To prevent this increase, screening for CKD and early intervention are necessary. In diabetic patients, the early detection of diabetic nephropathy has focused on the measurement of urinary albumin excretion rate. The elevated urinary albumin excretion rate within microalbuminuric level (30-299 mg/24 h or a spot urine albumin-to-creatinine ratio of 30-299 mg/g) allows the detection of patients with an increased risk for the development of overt diabetic nephropathy with persistent macroalbuminuria. Moreover, impaired renal function may be present even in patients with normal urinary albumin excretion rate [3]. Gold standard procedures for glomerular filtration rate (GFR) measurement, based on the clearance of 51Cr-EDTA or iohexol, are impractical in clinical settings and for larger research studies. Recently due to the high prevalence of diabetes and due to the improved therapeutic strategies, there has been a continuous increase in the incidence end-stage renal failure among patients with diabetes.

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Diabetic nephropathy is the commonest cause of end-stage renal disease in the world. This is mainly due to the increasing prevalence of type 2 diabetes mellitus. 1. It is characterized by microalbuminuria, subsequent macroalbuminuria, and declining GFR. However, there are patients with diabetics who have combination of normal albuminuria or microalbuminuria and impaired renal function , but not the traditional decline of GFR with the development of proteinuria.

2. Screening for diabetic nephropathy is currently done by measuring microalbuminuria, serum creatinine, and creatinine clearance (CCr). Serum creatinine is the most widely used marker of glomerular filtration rate in clinical practice, although it has low sensitivity in early renal disease. The serum creatinine level depends on muscle mass and meat in take, and its estimation may have positive interference from glucose, protein and fructose. Isotopic and non-isotopic methods for the determination of GFR, though accurate, are expensive and complex making them impractical for routine use. The other common method used is the creatinine clearance, a test that compares serum creatinine level with creatinine concentrations in a 24-hour urine collection [4].

Cystatin C, a cysteine protease inhibitor, is freely filtered by the renal glomeruli, metabolized by the proximal tubule and identified as a promising marker of renal failure [4]. Cystatin C is produced at a constant rate by nucleated cells and released into bloodstream with a half-life of 2 h. Its concentration is almost totally dependent on GFR. Other studies have demonstrated that serum cystatin C is an early renal marker in diabetic patients [5-7], but not all studies have done so [8]. Thus, we explored the possibility of the cystatin C levels of serum and urine as markers of early renal impairment in normoalbuminuric patients with diabetes. We also evaluated the relationship of albuminuria and serum/urine cystatin C.

2. Materials and Methods

2.1 Patients

We retrospectively studied the samples of serum and urine from 60 patients with type 2 diabetes who visited Tarnaka hospital of Hyderabad city in India between January 2016 and April 2016. The samples of serum and urine from 60 patients are shown in Fig. 1. We recorded confidential information of name, age, gender, race, height, weight and history of renin-angiotesin system inhibitors or antihypertensive medication. Because thyroid function could affect the levels of cystatin C [9], we excluded the patients with thyroid disease, or taking the medication due to thyroid disease in 6 months. We also excluded patients with uncontrolled hypertension making an effect on albuminuria.

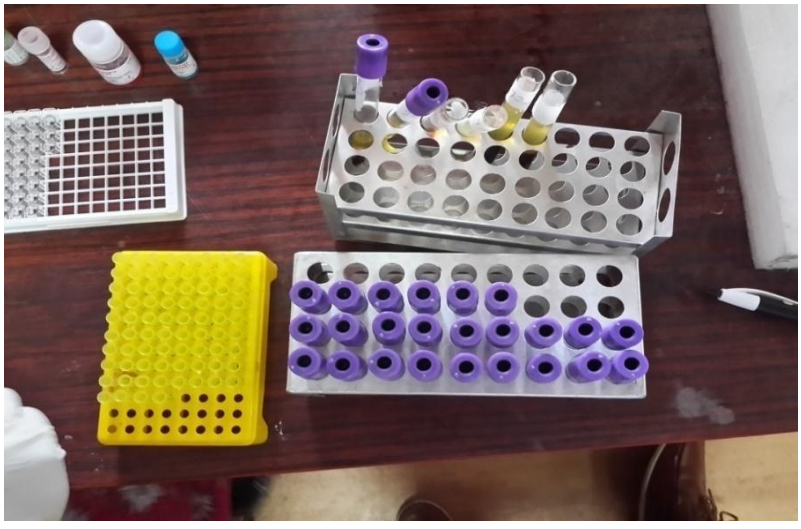

Fig. 1 The samples of serum and urine from 60 patients

The cystatin C levels of serum and urine were measured by the latex agglutination test (Modular P800, Roche, Diagnostics, Mannhein, Germany). The eGFR level was calculated using the modification of diet in renal disease (MDRD) formula: MDRD = 186 × (serum creatinine [mg/dL]) -1.154 × age - 0.203 [10]. A correction factor of 0.742 was used for women. The eGFRcys level was calculated by the chronic kidney disease epidemiology (CKD-EPI) equation: eGFR = 127.7 × (cystatin C in mg/L) - 1.17 × (age in years) - 0.13 × (0.91 if female) [11]. Patients were divided into 3 groups according to their urinary albumin concentration: those with normoalbuminuria (n = 210), those with microalbuminuria (n = 83) and those with macroalbuminuria (n = 42). Moreover, normoalbuminuric patients were subdivided according to eGFR calculated by the MDRD formula: those with ≥ 60 mL/min/1.73 m^2 (n = 181) and those with GFR < 60 mL/min/1.73 m^2 (n = 29) [6].

We have therefore reviewed the evidence base for cystatin C and its potential clinical utility as a marker of renal functions the methods and material were used diabetes induced by feeding high (65%) fructose rich diet to male SD rats weighing 180 to 200 gm for 8 weeks. Control rats were fed with (65%) cornstarch diet for the same duration. In three drug treated groups, resveratrol, nicotinamide and metformin were administered at a dose of 10 mg/kg (orally), 500 mg/kg (I.P) and 300 mg/kg (orally) for 8 weeks. Rats were sacrificed and liver tissues were collected from each animal, and stored for the estimation of all biochemical and molecular biology parameters. The human cystatin C ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human cystatin C in serum, plasma, cell culture supernatants and urine.

2.2 ELISA Plate Reader

ELISA stands for enzyme linked immunosorbent assay. In short, it is an antibody test or a test for immune response to things attacking the body such as virus, bacteria and allergens. The test is done in an ELISA plate, also known as a 96-well plate or micro plate. The ELISA reader reads the plate.

2.3 Analyzer Chem

Chemistry analyzers can be bench top devices or placed on a cart; other systems require floor space. They are used to determine the concentration of certain metabolites, electrolytes, proteins, and/or drugs in samples of serum, plasma, urine, cerebrospinal fluid, and/or other body fluids. Samples are inserted in a slot or loaded onto a tray, and tests are programmed via a keypad or bar code scanner. Reagents may be stored within the analyzer, and it may require a water supply to wash internal parts. Results are displayed on a screen, and typically, there are ports to connect to a printer and/or computer.

2.4 The Cystatin C Estimation Kit

The Human Cystatin C ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human cystatin C in serum, plasma, cell culture supernatants and urine.

2.5 Ethics Statement

The institutional review board of the Road Transport Corporation (RTC) Tarnaka hospital of Hyderabad city in India (IRB review exemption No. 0740-1289), approved the study.

3. Results

3.1 Patient Characteristics

Fructose feeding significantly (p < 0.01) increased hepatic TBARS and conjugated dienes levels, and significantly (p < 0.05) decreased hepatic glutathione (GSH) levels, superoxide dismutase (SOD) activity and ascorbic acid (Vitamin C) levels. Resveratrol treatment significantly (p < 0.01) decreased hepatic TBARS and conjugated dienes levels, and significantly

($p < 0.05$) increased hepatic superoxide dismutase (SOD) activity, glutathione (GSH) levels, catalase activity and ascorbic acid (Vitamin C) levels when compared to diabetic group. Nicotinamide administration significantly ($p < 0.05$) increased hepatic glutathione (GSH) levels, superoxide dismutase (SOD) activity, catalase activity and ascorbic acid (Vitamin C) levels but did not show any change in hepatic TBARS level, nitric oxide level, H2S level and GPX activity when compared to diabetic group. However, the standard drug Metformin administration showed significant ($p < 0.01$) decrease in conjugated dienes levels and significant ($p < 0.01$) increase in ascorbic acid (Vitamin C) levels when compared to diabetic group but no change in any other parameters. Our gene expression study showed that Vitamin C transporter, Slc23a1 expression was reduced in diabetic group but increased significantly ($p < 0.05$) after Resveratrol administration. Again, increased expression of SIRT4 in diabetic liver was significantly ($p < 0.05$) reduced by Resveratrol. In this cross sectional analytic study, 60 blood samples from patients who attended Road Transport Corporation (RTC) Tarnaka hospital of Hyderabad city in India have been examined in the hospital's library through the period from January 2016 to April 2016. All the 60 samples have been tested for nephropathy in type 2 diabetes patients [12].

All the human samples were divided in a group of two containing 20 males and 15 females in each group. Few of them are under diabetic medication and few insulin injections. Most of the list include age group above 55 years with less people of middle age. Few have diabetic family history, while the rest got due to life style and their dietary habits.

3.1.1 Differences in the Cystatin C Levels of Serum and Urine according to Albuminuria

The levels of cystatin C in serum showed stepwise increase with albuminuric levels ($p < 0.001$, $p = 0.013$, respectively) (Table 1, Fig. 2). Serum cystatin C was significantly different according to their albuminuria (normoalbuminuria vs. microalbuminuria, $p < 0.01$; microalbuminuria vs macroalbuminuria, $p < 0.001$; Normoalbuminuria vs. macroalbuminuria, $p < 0.001$) (Table 1, Fig. 2). The level of urine cystatin C also showed stepwise increase with albuminuric level (normoalbuminuria vs. microalbuminuria, $p < 0.05$; microalbuminuria vs. macroalbuminuria, $p < 0.001$; normoalbuminuria vs. macroalbuminuria, $p < 0.001$) (Table 2, Fig.3).

3.1.2 Parameters Related to the Cystatin C Levels of Serum and Urine in Diabetic Patients

The correlations between the log-transformed cystatin C levels of serum and urine and the albumin

Table 1 Characteristics of metabolic and laboratory parameters in patients with type 2 diabetes.

No	Age	Sex	DM Duration	Patient taken in DM	HTN	HEART disease	Family history	Physical examination	S.Noi
1	60	M	2 years	Oral	No	No	No	170H-78W	7205900
2	47	F	2 years	Oral	No	No	Yes	167H-81W	7205903
3	54	M	7 years	Oral	No	No	No	168H-70W	27217505
4	34	M	2 years	Insulin	No	No	Yes	167H-58W	21270400
5	66	F	2 years	Oral	Yes	Yes	No	170H-78W	3857300
6	37	M	3 years	Oral	Yes	No	Yes	147H-71W	236153
7	42	M	2 years	Oral	No	No	No	170H-80W	42765600
8	51	M	10 years	Oral	No	No	No	160H-67W	28131500
9	62	M	10 years	Insulin	No	Yes	Yes	152H-87W	30385001
10	57	F	5 years	Insulin	Yes	Yes	Yes	147H-67W	8755301
11	50	M	7 years	Oral	Yes	No	Yes	163H-95W	20052701
12	41	F	1 years	Oral	No	No	Yes	173H-50W	271900
13	53	M	32 years	Oral	No	Yes	No	160H-55W	20010300
14	63	F	4 years	Insulin	No	No	No	167H-65W	7765800

(table 1 continued)

No	Age	Sex	DM Duration	Patient taken in DM	HTN	HEART disease	Family history	Physical examination	S.Noi
15	31	F	4 years	Oral	Yes	No	No	168H-70W	236976
16	48	M	10 years	Oral	No	No	No	147H-56W	25077100
17	58	M	3 years	Oral	No	No	No	175H-75W	253177
18	43	F	25 years	Oral	Yes	Yes	No	160H-90W	361500
19	51	M	15 years	Oral	No	No	No	165H-56W	20268707
20	67	M	7 years	Insulin	No	No	No	184H-74W	2727001
21	60	F	4 years	Oral	No	Yes	Yes	174H-83W	5147400
22	52	M	3 years	Oral	No	No	No	153H-57W	4338100
23	62	M	35 years	Oral	Yes	No	No	160H-60W	300450
24	69	M	5 years	Insulin	No	No	Yes	159H-50W	2040500
25	66	M	2 years	Oral	No	Yes	No	165H-85W	30660902
26	45	M	10 years	Oral	No	Yes	No	165H-65W	5147400
27	58	M	4 years	Oral	No	Yes	No	167H-70W	4338100
28	78	F	6 years	Oral	No	No	Yes	160H-59W	300450
29	62	M	20 years	Oral	Yes	Yes	Yes	176H-75W	20401500
30	55	F	10 years	Insulin	Yes	No	Yes	149H-66W	30660902
31	68	F	3 years	Oral	No	No	No	179H-96W	21917403
32	38	M	10 years	Insulin	No	No	Yes	148H-55W	504148
33	29	M	30 years	Insulin	No	Yes	No	159H-55W	20400309
34	74	F	6 years	Oral	Yes	Yes	No	175H-75W	21751900
35	29	M	10 years	Oral	No	Yes	No	154H-70W	21485104
36	54	F	2 years	Oral	Yes	Yes	Yes	170H-60W	2268300
37	41	F	15 years	Insulin	No	No	Yes	171H-70W	21716301
38	53	F	10 years	Insulin	No	No	Yes	160H-70W	20064500
39	68	M	13 years	Insulin	No	No	Yes	174H-70W	29094801
40	71	M	2 years	Oral	No	No	No	176H-66W	80090900
41	62	F	4 years	Oral	No	Yes	No	147H-66W	4196400
42	48	M	2 years	Oral	Yes	No	No	169H-66W	11404602
43	62	M	16 years	Oral	No	No	No	158H-81W	27169400
44	65	M	12 years	Oral	No	Yes	Yes	170H-66W	30649900
45	33	F	18 years	Oral	Yes	No	No	168H-66W	9890800
46	63	F	4 years	Insulin	No	No	No	167H-65W	7765800
47	31	F	4 years	Oral	Yes	No	No	168H-70W	236976
48	54	F	2 years	Oral	Yes	Yes	Yes	170H-60W	2268300
49	41	F	15 years	Insulin	No	No	Yes	171H-70W	21716301
50	53	F	10 years	Insulin	No	No	Yes	160H-70W	20064500
51	42	M	2 years	Oral	No	No	No	170H-80W	42765600
52	51	M	10 years	Oral	No	No	No	160H-67W	28131500
53	62	M	10 years	Insulin	No	Yes	Yes	152H-87W	30385001
54	40	M	10 years	Oral	No	Yes	Yes	170H-60W	2268300
55	55	F	2 years	Oral	No	No	No	171H-70W	21716301
56	43	F	15 years	Oral	No	No	No	160H-70W	20064500
57	38	F	10 years	Oral	Yes	No	No	174H-70W	29094801
58	53	F	13 years	Oral	No	Yes	Yes	176H-66W	80090900
59	48	M	2 years	Oral	No	No	Yes	147H-66W	4196400
60	39	M	4 years	Insulin	Yes	No	Yes	169H-66W	11404602

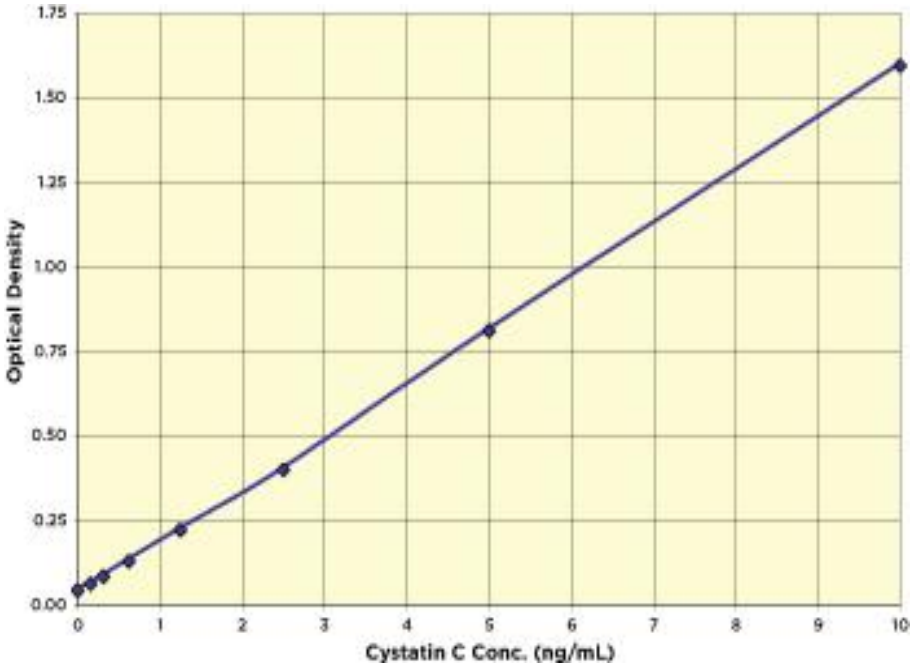

Fig. 2 these were the patient details of the samples collected by Hasan.

Table 2 to all the patients, by using cystatin C estimation kit the levels were determined and they have been mentioned below.

Sl. No.	FBS	PLPS	B.U	SC	FLP	HB	BP	HPA1C	Cystatin C
1	88	110	37	1.6	108	13.5	120/80	154	0.1
2	119	210	19	1.3	101	13	121/79	150	0.5
3	165	200	42	1.8	112	12.5	122/80	157	1.17
4	109	184	20	1.2	107	14	120/80	155	0.57
5	115	184	22	1.3	109	12.5	120/80	153	0.78
6	109	178	21	1.4	97	13.5	119/77	152	0.11
7	231	376	17	1.2	113	14.5	120/80	154	0.86
8	123	266	15	1.3	100	15	120/77	150	0.54
9	121	243	19	1.4	109	11.5	120/80	151	0.73
10	191	308	52	1.9	176	12	140/89	170	3.10
11	132	197	57	2.1	210	13.5	145/91	188	4.50
12	100	126	35	1.7	121	14	122/88	155	1.08
13	136	214	38	1.5	125	12.5	120/80	156	1.16
14	127	252	33	1.7	137	14.5	120/81	157	1.42
15	170	256	43	1.9	142	15	123/83	156	1.93
16	129	290	44	1.6	127	16	125/86	156	1.47
17	175	261	45	1.5	156	16.5	126/83	155	1.56
18	92	146	42	1.7	162	17	122/85	154	1.58
19	123	175	28	1.2	126	12	120/80	154	0.93
20	102	216	17	1.1	164	12.5	120/81	154	0.45
21	87	131	18	1.3	115	14	122/80	153	0.75
22	134	267	55	1.9	166	13.5	149/95	165	2.6
23	132	235	26	1.2	114	13	121/80	154	0.9
24	100	321	20	0.9	102	16	122/82	152	0.45
25	136	302	21	0.8	103	12	120/80	153	0.38
26	165	210	24	1.2	119	12.5	123/82	155	0.80
27	392	396	37	1.4	120	13	126/86	156	0.96

(table 2 continued)

Sl. No.	FBS	PLPS	B.U	SC	FLP	HB	BP	HPA1C	Cystatin C
28	137	189	39	1.41	122	14.5	125/84	155	0.98
29	96	105	25	0.9	116	12.5	120/80	154	0.52
30	156	179	46	1.7	143	15.5	139/86	155	1.56
31	89	100	32	1.1	141	16	127/80	156	0.57
32	95	120	19	0.92	112	13.5	121/81	154	0.46
33	88	121	17	0.95	113	14	120/80	153	0.52
34	87	117	20	0.89	108	12.5	120/80	152	0.40
35	105	116	43	1.6	141	13.5	131/80	155	1.3
36	103	107	22	1.2	111	16.5	129/80	154	0.6
37	100	105	18	0.87	107	15.5	125/83	153	0.47
38	94	98	22	0.97	115	15	123/80	154	0.59
39	196	215	56	1.95	167	13.5	145/85	169	2.47
40	86	95	23	1.2	105	14.5	121/80	155	0.55
41	236	321	54	2.5	175	16.5	146/90	188	3.5
42	125	217	47	1.5	146	15.5	139/86	158	1.47
43	116	149	41	1.2	109	13.5	120/80	154	0.95
44	107	134	19	1.3	112	14	121/80	154	0.89
45	127	156	42	1.3	137	13.5	132/85	156	1.47

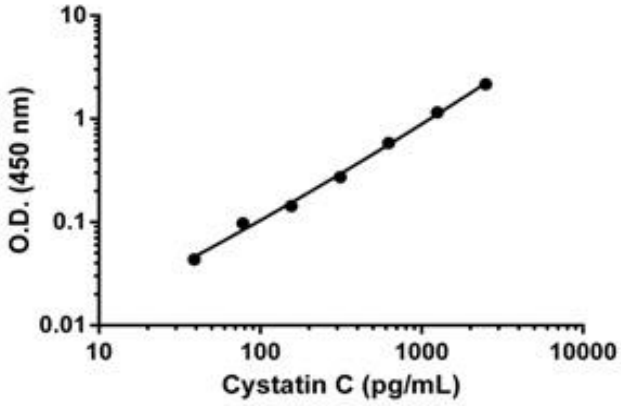

Fig. 3 Cystatin C has shown strong associations with GFR and albuminuria among patients with SCA and so may be a useful screening tool in this patient population.

creatinine ratio were analyzed in all diabetic patients. The serum level of cystatin C was found to directly correlate with albuminuria ($r = 0.555$, $p < 0.001$). The urine level of cystatin C also positively correlated with albuminuria ($r = 0.500$, $p < 0.001$). In Pearson's correlation analysis, the serum level of cystatin C was related to age, ACR, creatinine, eGFR, C-reactive protein (CRP), high-density lipoprotein and systolic blood pressure; and the urine level of cystatin C was related to ACR, HbA1C, creatinine, GFR, CRP and glucose. We performed a stepwise multiple regression analysis with these factors. The serum level of cystatin C was related to CRP, ACR and GFR, and the urine level of cystatin C was related to triglyceride, age, eGFR and ACR.

3.1.3 Differences in the Cystatin C Levels of Serum and Urine according to eGFR in the Normoalbuminuric Group

Table 2 presents the clinical characteristics of 210 patients with normoalbuminuria according to their eGFR. The patients with eGFR < 60 mL/min/1.73 m^2 ($n = 29$, 14%) by the MDRD equation were older, had lower high density lipoprotein levels (40.3 ± 12.2 vs. 46.9 ± 12.4 mg/dL, $p = 0.008$), had higher cystatin C

levels of serum (1.21 ± 0.42 vs. 0.86 ± 0.18 mg/L, $p < 0.001$) and urine (0.11 ± 0.11 vs. 0.06 ± 0.45 mg/L, $p = 0.013$) than those with eGFR ≥ 60 mL/min/1.73 m^2. However, there were no significant differences in ACR.

4. Discussion

In this study, we aimed at evaluating the cystatin C levels of serum and urine in a small cohort of patients with type 2 diabetes by categorizing them into 3 groups depending on their different degrees of kidney damage (normalbuminuria, microalbuminuria and diabetic nephropathy). In normoalbuminuric patients, the cystatin C levels of serum and urine were significantly increased in patients with GFR ≤ 60 mL/min/1.73 m^2 than those with GFR > 60 mL/min/1.73 m^2. It was thought that this increment was probably due to the tubular phase before glomerular manifestation. This suggests that the cystatin C levels of serum and urine are related to subclinical tubular impairment and can be earlier measurable markers of renal involvement before onset of albuminuria. In these patients, the cystatin C levels of serum and urine were independent factors to predict eGFR < 60 mL/min/1.73 m^2 estimated by the MDRD equation [13]. This finding indicated that the cystatin C could be an index reflecting renal tubular epithelial cells. With the EPI equation, the decreased level of high-density lipoprotein was the only independent factor to predict eGFR < 60 mL/min/1.73 m^2. This result is consistent with those of previous studies demonstrating that lipid metabolism may participate in the development of glomerular and tubular alterations, leading to nephron destruction. Our results suggest that dyslipidemia can be a risk factor for kidney damage in normoalubminuric diabetic patients. Further studies are needed to confirm these results.

Our study showed that serum cystatin C was associated with CRP, ACR and eGFR, whereas urine cystatin C was associated with TG, age, eGFR and ACR in the stepwise multiple regression analysis. A recent study has suggested the relationship between cystatin C and factors such as old age, male, overweight, CRP and inflammation [12, 13]. Our results are consistent with those studies.

The routine classical evaluation of diabetic nephropathy includes appearance of microalbuminuria, decreased creatinine clearance and increased serum creatinine [14]. However, it has been reported that a decline in the renal function of patients with diabetes was not always accompanied by an increased ACR [15, 16]. About 20%-30% of patients with type 2 diabetes, accompanied by renal insufficiency, showed normoalbuminuria [15-20]. To overcome these limitations, many clinicians additionally used creatinine in evaluating such patients. However, serum creatinine also depends on creatinine production, extrarenal elimination and tubular handling [17]. Moreover, tubular involvement may precede glomerular involvement because several tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and a rise in serum creatinine [18, 19]. Therefore, other biomarkers for estimation of renal function have been searched for and one of them was cystatin C [20]. Our study results confirmed that cystatin C could be one of the additional tubular factors, which represent kidney state of diabetic patients.

This study has some limitations. First, owing to the retrospective cross sectional design, it was difficult to clarify the causal relationship between the risk factors and the natural course of normoalbuminuric renal insufficiency [21, 22]. Moreover, the patients with normoalbuminuria and eGFR < 60 mL/min/1.73 m^2 might need more evaluation such as kidney biopsy to diagnose diabetic nephropathy. Second, eGFR, estimated by the MDRD or EPI-equation, did not appear to reflect actual kidney function. Therefore, we could not conclude which factor is more accurate or useful. Third, the subjected patients were not asked to discontinue their medications, such as antihypertensive medications. Therefore, albuminuria might be underestimated in these patients. Nevertheless, this

study has some strength. We evaluated the levels of cystatin C in both serum and urine at the same time. In addition, this study demonstrated clearly that the cystatin C levels of serum and urine were increased along with the level of albuminuria in diabetic patients.

5. Conclusion

Diabetes is a well-known disease of the 21st century, which is having no effective treatment till now. Liver disease is an important cause of death in type 2 diabetes apart from various other cardiovascular, retinal, neural, skeletal complications. This study mainly deals to assess the effectiveness and appraise the diagnostic value of cystatin C as biomarkers for early detection of nephropathy in type 2 diabetes patients. Diabetic nephropathy (DN) is one of the most serious micro vascular complications of diabetes. The accumulation of the extracellular matrix proteins, like laminin, fibronectin or type-IV collagen is one of the major characteristics of the early diabetic nephropathy (DNP) by estimating the diagnostic value of serum neutrophil gelatinase-associated lipocalin (NGAL) and retinol-binding protein 4 (RBP4) as biomarkers for early detection of nephropathy in type 2 diabetic patients. Screening for diabetic nephropathy is currently done by measuring microalbuminuria, serum creatinine, and creatinine clearance (CCr). Serum creatinine is the most widely used marker of glomerular filtration rate in clinical practice. GFR used serum creatinine and serum cystatin C based predictive equations and [3] association of diabetic kidney disease covariates like hypertension, retinopathy, lipid levels and coronary artery disease (CAD). The first step in the screening and diagnosis of diabetic nephropathy is to measure albumin in a spot urine sample, collected as the first urine either in the morning or at random, for example, at the medical visit. This method is accurate, easy to perform, and recommended by American Diabetes Association guidelines. GFR is the best parameter of overall kidney function and should be measured or estimated in micro- and macro-albuminuric diabetic patients. In micro-albuminuric patients when the kidneys are functioning normally, concentrations of cystatin C in the blood are stable, but as kidney function deteriorates, the concentrations begin to rise. Because cystatin C levels fluctuate with changes in GFR, there has been interest in the cystatin C test as one method of evaluating kidney function. Due to the many problems encountered with measurements of creatinine and its use as a GFR estimate, cystatin C has been proposed as an alternative marker of renal function. The potential utility of serum cystatin C in the laboratory lies in its capability to detect early renal failure, i.e. at stage 2 CKD (i.e. GFR level of 60 to 90 mL/min/1.73 m^2). The study aims to evaluate the diagnostic value of serum neutrophil gelatinase-associated lipocalin (NGAL) and retinol-binding protein 4 (RBP4) as biomarkers for early detection of nephropathy in type 2 diabetic patient. Maintaining blood glucose levels, blood pressure, and cholesterol at or close to normal can help delay or prevent diabetes complications. Therefore, people with diabetes need regular monitoring and avoiding complications thus leading a healthy life. In conclusion, the results of this study suggest that cystatin C measurement in urine and serum is a useful, practical, non-invasive tool for the evaluation of renal involvement in the course of diabetes, especially in normoalbuminuric patients. Further investigations with a larger sample size and a prospective design are required to confirm the potential application of cystatin C as a useful biomarker for the early detection of diabetic nephropathy.

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