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 m² estimated by MDRD equation in patients with normoalbuminuria. On the other hand, eGFR < 60 mL/min/1.73 m² 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.
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-angiotensin 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 m² (n = 181) and those with GFR < 60 mL/min/1.73 m² (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 barcode 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            | 28131300 |
| 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  |
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(Continued)

| No | Age | Sex | DM Duration | Patient taken in DM | HTN | HEART Disease | Family history | Physical examination | S.No |
|----|-----|-----|-------------|---------------------|-----|---------------|----------------|---------------------|------|
| 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           | 21845104 |
| 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  | No            | 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 |
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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       |
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.

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 $eGFR < 60 \text{ mL/min/1.73 m}^2$ ($n = 29$, 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 m². 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 (normal albuminuria, 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 m² than those with GFR > 60 mL/min/1.73 m². 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 m² 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 m². 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 normoalbuminuric 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 m² 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 microvascular 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 m²). 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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