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Original Article

Serum cystatin C as an indicator for early detection of diabetic nephropathy in type 2 diabetes mellitus

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ABSTRACT

Background: Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. The metabolic dysregulations associated with DM causes secondary pathophysiological changes in multiple organ systems which result in various complications, responsible for the morbidity and mortality associated with the disease.
Methods: The present study was carried out on 40 patients with type 2 diabetes mellitus, who were recruited from those attending outpatient clinic and inpatient of Internal Medicine Department at The National Institute of Diabetes and Endocrinology from January 2017 to june 2017.
Results: The mean Cystatin C values in Group I were 0.74, group II were 1.07. and in Group III were 3.25, The results show that the Cystatin C values were raised even in the patients with Normoalbuminuria with GFR ≥90 whom clinical albuminuria had not yet started.
Conclusions: serum Cystatin C may be considered as an early marker, than microalbuminuria and serum creatinine, the commonly used marker for nephropathy, for declining renal function, in diabetic subjects. Further studies in larger population are needed to confirm this result.

1. Introduction

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycemia. The metabolic dysregulations associated with DM causes secondary pathophysiological changes in multiple organ systems which result in various complications, responsible for the morbidity and mortality associated with the disease [1]. The estimated prevalence of T2DM world-wide for 2017 has risen to 404.7 million, and the total number of people with diabetes is expected to rise to 679.7 million by 2045. In 2017, approximately 38.7 (27.1–51.4) million people, or 9.6% (6.7–12.7) of adults aged 20–79 years are living with diabetes in MENA. About 49.1% of these are undiagnosed [2]. Diabetic kidney disease, or kidney disease attributed to diabetes, occurs in 20–40% of patients with diabetes and is the leading cause of end-stage renal disease (ESRD) [3]. Screening for kidney damage (albuminuria) can be most easily performed by urinary albumin–to–creatinine ratio (UACR) in a random spot urine collection. Timed or 24-h collections are more burdensome and add little to prediction or Accuracy [4], [5]. Measurement of a spot urine sample for albumin alone (whether by immunoassay or by using a sensitive dipstick test specific for albuminuria) without simultaneously measuring urine creatinine (Cr) is less expensive but susceptible to false-negative and false positive determinations as a result of variation in urine concentration due to hydration. Normal UACR is defined as, 30 mg/g Cr, and increased urinary albumin excretion is defined as 30 mg/g Cr. Because of variability in urinary albumin excretion, two of three specimens of UACR collected within a 3- to 6-month period should be abnormal before considering a patient to have albuminuria. Exercise within 24 h, infection, fever, congestive heart failure, marked hyperglycemia, menstruation, and marked hypertension may elevate UACR independently of kidney damage. Estimated Glomerular Filtration Rate Serum Cr should be used to estimate glomerular filtration rate (GFR). Estimated GFR (e GFR) is commonly reported by laboratories or can be estimated using formulae such as the Modification of Diet in Renal Disease (MDRD) study equation [6]. Persistently increased UACR in the range of UACR 30–299 mg/g Cr is an early indicator of diabetic kidney

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disease in type 1 diabetes and a marker for development of diabetic kidney disease in type 2 diabetes. It is also a well-established marker of increased CVD risk [7,8]. Not all people with diabetes, kidney disease, and reduced eGFR have albuminuria. In addition, there is increasing evidence that up to 40% of patients with type 1 diabetes and UACR levels 30–299 mg/g Cr have spontaneous remissions and approximately 30–40% remain with UACR levels of 30–299 mg/g Cr and do not progress to higher levels over 5–10 years of follow up [9]. Patients with persistent and severely increased (300 mg/g Cr) levels of albuminuria are likely to develop ESRD [10,11]. Physical activity can acutely increase urinary protein excretion. However, there is no evidence that vigorous-intensity exercise increases the rate of progression of diabetic kidney disease, and there appears to be no need for specific exercise restrictions for people with diabetic kidney disease [12]. Cystatin-C Formed of a 122-amino acid, 13-kDa protein that is a member of the family of cysteine proteinase inhibitors [13]. It is encoded by the 'housekeeping type" CST3 gene, and produced by all nucleated cells at a constant rate [14]. It is freely filtered by the glomerulus and catabolized primarily by proximal tubular cells [15]. Cystatin C is found in the systemic circulation with high concentrations and it inhibits cysteine proteases extracellularly also it has a significant antiviral activity. Its concentration in the CSF is 5.5-times higher than in the serum. Non negligible levels are also found in saliva and sperm [16]. Cystatin C seems to be a promising candidate as a novel marker of the GFR [17]. It has been suggested that Cystatin C could be especially useful in the detection of early nephropathy, as demonstrated by the increased cystatin C level in patient with microalbuminuria, but with normal GFR [18].

2. Subjects and methods

This cross-sectional prospective study was conducted on 40 subjects, 10 healthy volunteers and 30 Diabetic patients. It was conducted on males and non pregnant females with their ages ranging from 34-63 years old who were recruited from those attending outpatient clinic and inpatient of Internal Medicine Department at The National Institute of Diabetes and Endocrinology from January 2017 to june 2017. Diabetic patients were classified into 3 groups according to A/C ratio in urine: Group I (A1): Consist of 17 patients (5 males 12 females) with diabetic normoalbuminuria, urinary (A/C) $\leq$ 30 mg/g. Group II (A2): Consist of 13 patients (6 males 7 females) with diabetic micro-albuminuria, urinary (A/C) 30–300 mg/g. Group III (A3): Consist of 10 patients (4 males 6 females) with diabetic macro-albuminuria, urinary (A/C) $\geq$ 300 mg/g.

3. Study design

Thorough history and clinical examination was performed to every patient with special stress on age, sex, body mass index, smoking state, type and duration of diabetes,medications, complications, other co-morbidities, ECG, Abdominal U.S and Laboratory tests were done to every subject as (fasting & postprandial plasma glucose level AST,ALT,S. Albumin, Total Protein, CBC, HbA1C, serum creatinine, blood urea, estimated GFR by MDRD formula, serum TSH, serum Cystatin C level using the ELISA technique).

4. Statistical analysis

All collected data were tabulated and analyzed using SPSS (Statistical Package for social science) version 16 to obtain:

5. Descriptive data

Descriptive statistics were calculated for the data in the form of:

1. Mean and Standard deviation (±SD) for quantitative data.
2. Frequency and distribution for qualitative data.

6. Analytical statistics

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests.

1. Student's *t*-test: used to compare mean of two groups of quantitative data.
2. ANOVA test (F value): used to compare mean of more than two groups of quantitative data.
3. Inter-group comparison of categorical data was performed by using fisher exact test (FET).
4. Correlation coefficient: to find relationship between variables.
5. A P value < 0.05 was considered statistically significant (*) while > 0.05 statistically insignificant.

7. Results

The age of the studied groups ranges from 33 to 67 years with the mean age 52 years of which 20 were males (37.5%) and 30 were females (62.5%) [Table 1], BMI ranged from 22(Kg/m2) to 44(Kg/m2) with the mean BMI of 33.5(Kg/m2). This study shows that BMI is significantly high in group I-(A1) compared to other groups [Table 3], There was a high significant difference in FBG ratio and in 2HPP among all the studied groups. It was found that (HbA1c) significantly higher in macroalbuminuric group than other groups and microalbuminuric group is higher than normoalbuminuric group [Table 4]. There was statistically high significant difference among normo, micro and macroalbuminiric diabetic group as regard the mean value ± SD of DM duration [Table 2]. There was insignificant difference between the four studied groups regards TSH [Table 5]. There was high significant difference between the studied groups regarding S. creatinine, BUN and eGFR [Table 6]. The study showed high significant difference between the diabetic group and control group regarding S. Cystatin C Also the mean value of serum Cystatin C was significantly high in Macroalbuminuric group compared to other groups (mean = 3.25).[Table 7]. There was significant negative correlation between S. Cystatin C and eGFR Also there was significant positive correlation between Serum cystatin c and s. Creatinine, Blood. urea. A/C ratio in urine, HbA1c, FBG, 2Hpp and DM duration. On the other hand there was insignificant correlation between serum cystatin C in relation to Age[Table 8]. The study showed that there was a significant difference when comparing control group and patient with normoalbuminuria with GFR $\geq$90 in relation to serum Cystatin C [Table 9]. Linear regression showed that S. Cystatin C is a significant predictor for eGFR and A/C ratio in urine (see Table 10) [Table 11].

8. Discussion

The development of microalbuminuria and the progression to overt proteinuria are the most common clinical features. However, in contrast to the predictions of the classical model for kidney disease involvement, a considerable percentage of patients with diabetes and impaired renal filtration do not have substantially elevated urinary protein excretion rates. All studies on this subject

Table 1
Comparison between the diabetic patients and control group regarding socio-demographic characters.

Variable		Control group (n = 10)		Diabetic group (n = 40)		Test of sig	P
Age (ys)	Mean ± SD Range	48.9 ± 8.8 34–63		52.0 ± 7.7 33–67		St."t" = 1.1	0.27 (NS)
		No.	%	No.	%		
Sex	Male Female	5 5	50.0 50.0	15 25	37.5 62.5	FET	0.49 (NS)

FET→ Fisher's Exact test.

Table 2
Comparison between the different diabetic groups regarding duration of DM.

Group	n.	Duration of DM (ys)			ANOVA	P	Bonferroni test for sig. pairs	P
		Mean	± SD	Range				
Normoalbuminuria (A1)	17	5.47	3.46	1–12	18.3	<0.001 (HS)	Normo ≠ Micro	0.31
Microalbuminuria (A2)	13	7.23	2.24	4–11			Normo ≠ Macro	<0.001
Macroalbuminuria (A3)	10	12.30	2.35	8–16			Micro ≠ Macro	<0.001

Table 3
Comparison between the four studied groups regarding BMI.

Group	n.	BMI (kg/m^2)			ANOVA	P
		Mean	± SD	Range		
Control gr.	10	27.0	2.58	23–31	8.5	<0.001 (HS)
Normoalbuminuria (A1)	17	39.9	9.07	25–54		
Microalbuminuria (A2)	13	34.0	5.78	23–41		
Macroalbuminuria (A3)	10	31.0	6.21	22–44		

Bonferroni test for sig pairs

Significant pairs	P
Controls ≠ Normo	<0.001 (HS)
Controls ≠ Micro	0.11
Controls ≠ Macro	1.0
Normo ≠ Micro	0.13
Normo ≠ Macro	0.011 (S)
Micro ≠ Macro	0.98

are observational and most lack biopsy data. A well-designed biopsy study and a series of intervention trials are needed to fully understand this entity [19]. With regard to this aspect, Tervaert et al. reported in 2010 a new pathology classification of the diabetic kidney lesions where the authors insisted on the existence of some forms of kidney damage with primary involvement of tubules, interstitium and/or the vessels, far away from the classical nodular or global glomerulosclerosis [20]. There is also evidence of an increase in systemic and vascular markers of inflammation as the size of the kidney progressively increases. Accompanying these changes are abnormalities in the blood biochemical indices of renal function, which precede renal failure [21]. The GFR is considered the most accurate measurement of kidney disease and is reduced before the onset of clinical symptoms, GFR is measured or predicted according to different methods [22]. There is no simple and practical way to measure GFR directly, so it is estimated. To estimate the

Table 4
Comparison between the four studied groups regarding parameters of glycemic control.

Variable	Control group (n = 10)			Normo albuminuria group (n = –17)			Micro albuminuria group (n = 13)			Macro albuminuria (n = 10)			ANOVA	P
	Mean	±SD	Range	Mean	±SD	Range	Mean	±SD	Range	Mean	±SD	Range		
FBS (mg/dl)	85.4	8.88	73–96	130.1	20.63	81–163	221.9	58.49	135–305	229.4	56.35	143–355	33.3	<0.001 (HS)
2 h PP	113.0	9.94	100–126	186.1	42.08	114–266	301.7	82.84	175–441	386.8	81.44	246–516	42.5	<0.001 (HS)
HbA1c (%)	5.03	0.52	4.2–5.7	7.00	1.22	5.9–9.8	7.90	0.98	6.6–10.1	9.37	1.93	6.7–12.6	21.5	<0.001 (HS)

Bonferroni test for significant pairs

Pairs	P (FBS)	P (2h PP)	P (HbA1c)
Controls ≠ Normo	0.052	0.025	0.002
Controls ≠ Micro	<0.001	<0.001	<0.001
Controls ≠ Macro	<0.001	<0.001	<0.001
Normo ≠ Micro	<0.001	<0.001	0.33
Normo ≠ Macro	<0.001	<0.001	<0.001

Table 5
Comparison between the studied groups regarding TSH.

Variable	Control group (n = 10)			Normo albuminuria group (n = –17)			Micro albuminuria group (n = 13)			Macro albuminuria (n = 10)			ANOVA	P
	Mean	±SD	Range	Mean	±SD	Range	Mean	±SD	Range	Mean	±SD	Range		
TSH	2.39	0.95	0.49–3.82	2.78	1.24	0.72–4.63	2.93	0.97	1.36–4.16	2.42	1.51	0.49–4.74	0.58	0.63 (NS)

Table 6
Comparison between the studied groups regarding kidney function test.

Variable	Control group (n = 10)			Normo albuminuria group (n = −17)			Micro albuminuria group (n = 13)			Macro albuminuria (n = 10)			ANOVA	P
	Mean	±SD	Range	Mean	±SD	Range	Mean	±SD	Range	Mean	±SD	Range		
S. creat	0.88	0.21	0.6–1.2	1.30	0.57	0.5–2.4	1.59	0.42	1–2.5	2.47	1.35	0.6–5.0	8.7	<0.001 (HS)
Blood urea	30.5	6.50	21–40	60.5	19.90	28–85	81.2	12.22	65–110	105.6	19.32	75–136	40.9	<0.001 (HS)
eGFR	99.0	36.77	90.1–154	65.3	35.66	22.4–138.8	42.6	11.65	28.3–62.4	29.1	14.46	11–56	12.3	<0.001 (HS)

Bonferroni test for significant pairs

Pairs	P (creat)	P (BUN)	P (ACR)	P (eGFR)
Controls ≠ Normo	0.89	<0.001	1.0	0.025
Controls ≠ Micro	0.14	<0.001	<0.001	<0.001
Controls ≠ Macro	<0.001	<0.001	<0.001	<0.001
Normo ≠ Micro	1.0	0.007	<0.001	0.19
Normo ≠ Macro	0.001	<0.001	<0.001	0.013
Micro ≠ Macro	0.038	0.005	<0.001	0.93

Table 7
Comparison between the studied groups regarding serum cystatin.

Group		Serum cystatin			KWT	P
		Mean	± SD	Range		
Control gr.	10	0.65	0.20	0.37–0.91	29.8	<0.001 (HS)
Normoalbuminuria	17	0.74	0.22	0.35–1.12		
Microalbuminuria	13	1.07	0.31	0.45–1.64		
Macroalbuminuria	10	3.25	1.09	1.14–5.05		

Bonferroni adjusted Mann Whitney test for significant pairs for Cystatin

Pairs	P (0.008)
Controls ≠ Normo	1.0
Controls ≠ Micro	0.44
Controls ≠ Macro	<0.001
Normo ≠ Micro	0.50
Normo ≠ Macro	<0.001
Micro ≠ Macro	<0.001

Table 8
Correlation coefficient (r) between cystatin C and different parameters in the studied groups.

	serum Cystatin C	
	R	P
eGFR (ml/min/1.73 m^2)	−0.415	0.003
S.Creatinine (mg/dl)	0.466	0.001
Blood Urea (mg/dl)	0.599	0.000
AC ratio in urine	0.857	0.000
BMI (kg/m^2)	−0.183	0.204
HBA1C (%)	0.507	0.000
2HPP (mg/dl)	0.655	0.000
FBG (mg/dl)	0.444	0.001
Age (years)	0.153	0.289
DM duration (years)	0.692	0.000

GFR, an endogenous substance in the blood that is cleared by the kidney is used, this substance is currently serum creatinine, the Cockcroft-Gault (CG) and Modification of Diet in Renal Disease (MDRD) Study equations are serum creatinine-based equations that are used to estimate GFR, GFR determinations by creatinine-based equations are not precise, so other substances, such as cystatin C, are being explored to estimate GFR [23]. The primary limitation of creatinine is that levels are determined not only by GFR, but also by muscle mass and dietary intake, lower serum creatinine levels may less reliably detect impaired GFR in patients with certain characters like older age, female sex, chronic illness with muscle wasting, amputation, or a vegetarian diet, higher serum creatinine levels are associated with African American race, muscular body habitus, and a high protein diet, while estimating equations attempt to adjust for these factors, the result is not precise, different patients can have the same serum creatinine with very different GFR [24]. Creatinine clearance requires a 24 h urine collection, a blood sample is drawn during the 24 h period, and creatinine clearance, can then be calculated, there are several factors that may interfere with the accuracy of the test such as: incomplete urine collection, pregnancy, vigorous exercise and drugs (such as: cimetidine, trimethoprim) which compete with creatinine secretion or drugs that can damage the kidneys, therefore any result may be inaccurate [25]. Several new biochemical markers have the potential to be

Table 9
Comparison between control group and patient with normoalbuminuria with GFR ≥90 in relation to serum Cystatin C.

Group	N	Serum cystatin			MWU	P
		Mean	± SD	Range		
Control gr.	10	0.65	0.20	0.37–0.91	2.12	0.034 (S)
Normoalbuminuria with GFR ≥90	4	0.87	0.13	0.75–1.12		

Table 10
Stepwise multiple linear regression analysis for the predictors of GFR (nephropathy) among type 2 diabetic patients.

Model summary	R^2	Adjusted R^2	SEE	F	P-value
	0.367	0.351	23.4	22.06	<0.001 (HS)

Variable	Unstandardized Coefficients		Standardized Coefficients	95% CI of B	T	P
	B	Std. Error	Beta			
(Constant)	81.3	7.8		65.5–97.2	10.3	<0.001 (HS)
Serum Creat	−19.2	4.08	−0.606	−27.4-(-10.9)	4.69	<0.001 (HS)
Serum Cystatin C	−11.3	3.7	−0.456	−22.1- (−6.9)	2.71	0.007 (S)

eGFR = 81.3−19.2 (Serum creat) −11.3 (serum cystatin C).

markers of CKD progression, these new markers might reflect the early diminished glomerular filtration than the traditional markers, these include: Kidney Injury Molecule-1 (KIM-1), N -acetyl-β-glucosaminidase, β2 microglobulin, α1-microglobulin, and retinol binding protein, human neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), clusterin, fatty acid binding protein, and cystatin C [26]. Cystatin C is a low-molecular weight proteins (13kd) produced by all nucleated human cells, with a stable production rate. It is freely filtered by the glomerulus and catabolized primarily by proximal tubular cells. Cystatin C seems to be a promising candidate as a novel marker of the GFR [17]. It has been suggested that cystatin C could be especially useful in the detection of early nephropathy, as illustrated by the increased cystatin C level in patient with microalbuminuria, but with normal GFR [18]. Cystatin C is considered as a good marker of GFR as it not influenced by gender, muscle mass, age, protein intake, cystatin C is preferable to creatinine and creatinine clearance as there is no urine collection, increased sensitivity to even slight impairment of glomerular filtration and already increases significantly in the creatinine blind range, which enable early detection and treatment of CKD, filtered solely by the glomerulus, generated at a constant rate by all cells in the body, completely reabsorbed by the tubules and then catabolized and not secreted by the renal tubules [27]. In our study, the level of cystatin C was significantly higher than the normal level in patients groups when compared with control group [Table 7]. These results were in accordance with Uslu et al. who found that serum cystatin C was significantly higher in patients with diabetes mellitus than control group [28]. Although serum creatinine has became the most popularly used serum marker of renal function, serum creatinine may be unreliable because it is frequently affected by muscle mass, age, gender, and aberrant renal tubular regulation of serum creatinine resulting in an overestimation of GFR. Using serum cystatin C levels has some advantages over serum creatinine and creatinine-based calculated GFR formulas, in that serum cystatin C levels are independent of age, gender, muscle mass, and renal tubular secretion [29]. Our study showed that serum cystatin C was not affected by age [Table 1]. This data matched with [15] who found that measuring of cystatin C is not affected by the age. This may reflect the importance of cystatin C to use as renal function test in elderly or even in very young patients. Also.Akyhse-Andersen et al. confirmed that serum cystatin C concentrations were uninfluenced by age [30]. In contrast, [31] reported that there was significant positive correlation noticed between serum cystatin C and age. This controversy could be explained as the fact that serum cystatin C is thought to be produced at a constant rate by most nucleated cells and this rate may be affected by aging, apoptosis and cells damage. In our study Cystatin C was not affected by gender[Table 1]. Also, [32] and [33] reported that gender has no significant effect on Serum Cystatin C. Akyhse-Andersen et al. confirmed that serum cystatin C concentrations were uninfluenced by gender [30]. However, Knight et al., 2004 reported that serum cystatin C is influenced by non-renal factors such as age, gender, weight, height [34]. Although, the serum cystatin C level has been proven to be independent of gender, infections, dietary factors and liver diseases, but some suggest it is related to age, and is slightly higher in males [35]. [36] results indicated that the mean value of cystatin C for women was lower, and the difference was smaller than those for creatinine. This could be explained as higher weight and greater height and male gender are associated with raised plasma creatinine concentrations which in turn would tend to result in a lower calculated creatinine clearance since plasma cystatin c is adjusted for creatinine clearance. Cystatin c would appear to be raised in these situations [37]. Regarding BMI, in our study, there was statistically significant difference between the patients groups and the control group [Table 3]. The same result was reported by Refs. [38] and [39] have reported a significant correlation between serum cystatin C and BMI. In contrast, [40] who have reported a moderate but biologically insignificant correlation between BMI and cystatin C·In our study, all patients groups and control group were euthyroid with normal level of TSH. [41] and [42] reported that the production of cystatin C is differently influenced by thyroid hormones, even in mild forms and increased in hyperthyodism. There is association between poor glycemic control and DM duration with the development of DN, the diabetic patients with normoalbuminuric group, microalbuminuric group and macroalbuminuric group showed higher mean values of HbA1c when compared to control group. HbA1c means in the different diabetic groups were 7.0%, 7.9%, and 9.37% respectively [Table 4]. These

Table 11
Linear regression between (serum Cystatin C) in relation to (GFR and A/C ratio in urine).

Model		Unstandardized Coefficients		Standardized Coefficients	T	R^2	P value
		B	Std. Error	Beta			
GFR	(Constant)	*74.035*	*7.521*		*9.843*		*0.000*
	Cystatin C	*−13.064*	*4.968*	*−0.355*	*−2.629*	*0.126*	*0.011*
A/C ratio	(Constant)	*−41.837*	*33.654*		*−1.243*		*0.220*
	Cystatin C	*215.910*	*22.231*	*0.814*	*9.712*	*0.663*	*0.000*

GFR = 74.035−13.064(Cystatin C).
A/C ration in urine = −41.837 + 215.910(Cystatin C).

results were in accordance with Konrad Walczak et al. who stated that there is positive correlation between cystatin C and HbA1c [43]. In our opinion, poor glycemic control acts as inflammatory factor in the kidney, stimulate PKC and oxidative stress that enhancing expression of cytokines that attract more inflammatory cells to the kidney leads to endothelial dysfunction and microalbuminuria appear. Glomerular structural changes typical of diabetic nephropathy are commonly established by the time microalbuminuria becomes apparent [44] and [45]. In contrast, [46] observed there was non-significant correlation between cystatin C and glucose level indicating that serum cystatin C levels are independent of blood glucose level. In our study, there was a statistically significance difference between patients groups and control group regarding Blood Urea ($P < 0.001$) and it was higher in micro and macroalbuminuric groups than in normoalbuminuric group [Table 6]. Blood Urea is not an accurate marker for measuring renal function. It is filtered by glomerulus and reabsorbed by the renal tubule. Concentration of urea in serum could be vary with diet, also can increased in dehydration associated with poor glycemic control that cause polyuria. Regarding serum creatinine, in our study there was a statistically high significant difference between patients groups and control group ($P < 0.001$) [Table 6]. National Kidney Foundation and ADA recommend to measure serum creatinine once per year mainly to detect chronic kidney disease [47]. In contrast to our results [48], showed significant decreases in serum creatinine concentrations among diabetics compared to controls. These results may be explained based on glomerular hyperfiltration that may develop at initial stages of the DN. Our study showed that glycemic state has a significant impact on serum cystatin C level, this could be inferred that there was a positive significant correlation between Hba1C, FBG, 2hpp with serum Cystatin C [Table 8] As classical evaluation of DN includes appearance of microalbuminuria, decreased creatinine clearance and increased serum creatinine. It has been reported that a decline in the renal function of patients with diabetes was not always accompanied by an increased ACR. 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. Therefore, other biomarkers for estimation of renal function have been searched for, and one of them can be cystatin C·In our study, we used MDRD equation for measuring eGFR. There was high significance difference between the patients groups and control group ($P < 0.001$) and eGFR was lower in the groups of micro and macroalbuminuria than in normoalbuminuric group ($P = 0.025$, <0.001 respectively)[Table 6].The current Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines advocate creatinine based equations for estimating GFR to identify patients with potential kidney disease and to classify them into different stages on the basis of these results. These stages also include individuals with normal or near-normal GFR. Such stratification requires an accurate and precise measurement of GFR that is inexpensive, reliable, and widely available [49]. Validation of the MDRD formulas has been attempted in individuals with known renal disease and normal serum creatinine levels, patients with early DN, and individuals without kidney disease [48]. As cystatin C is produced at a constant rate by nucleated cells and released into the blood stream with a half-life of ~2 h and is freely filtered and almost completely taken up and degraded, but not secreted, by proximal tubular cells, so with the occurrence of pathological changes in diabetic kidney the filtration capacity is decreased with subsequent retention of cystatin C and increased serum level. The result of previous studies of the role of cystatin C in detecting early renal failure in diabetic patients were contradictory. Some authors showed that cystatin C was more effective than creatinine in detecting initial reduction of GFR in T2DM as [50] [51], and [52] showed that serum cystatin C was more sensitive than serum creatinine for estimation of GFR in T2DM patients. In contrast to these result [53], found that serum creatinine was better than serum cystatin C for the estimation of GFR in microalbuminuric and proteinuric diabetic patients. Oddoze et al. selected a heterogeneous group of type 1 and type 2 diabetic patients. In our study, there was statistical significant of serum cystatin C in patients groups in comparison to control group ($P < 0.001$), but there was no statistical significant difference between normoalbuminuric group in comparison to control group ($P = 0.1$) [Table 7]. The mean value of the cystatin C was significantly higher in the macroalbuminuric group compared to normoalbuminuric group, and microalbuminuric group [Table 7]. In this study we observed a significant increase in the level of cystatin C in patients with diabetic nephropathy compared to control group [Table 1], and this agrees with the results detected by Refs. [54, 55] and [53] who concluded in their studies that the diagnostic accuracy for cystatin C as shown by the AUCs of their ROC curves is comparable, in contrast to plasma creatinine, which has lower accuracy .[31] also demonstrated that serum cystatin C is better to detect mild diabetic nephropathy. [56] showed a significant relationship between serum cystatin C levels and the prognostic stage in patients with type 2 diabetic nephropathy. [57] showed that sensitivity of serum cystatin C is higher in microalbuminuric patients with diabetes. On the other hand [53], demonstrated that sCr and serum cystatin C is equal in diagnostic accuracy in microalbuminuric and proteinuric diabetic patients. In this study we used cut-off level of GFR (more than 90 ml/min)and there was significant correlation in normoalbuminuric group with GFR>90 in comparison to control group regarding Cystatin C ($p = 0.034$) [Table 9]. In addition, the American Diabetes Association applied the CKD staging to diabetic nephropathy [47]. Therefore, we studied the level of serum cystatin C using the FDA approved method in diabetic patients and analyzed the results in view of the markers frequently and routinely used in clinical practice, such as serum Cr and calculated GFR using (MDRD) equation. Our study results showed similar results compared to the previous studies, despite the changes in GFR classification. Both serum cystatin C and serum creatinine revealed a significant difference in the CKD staging group. In the CKD staging group, the mean value of cystatin C was significantly high in group 3 compared to group 1 and 2. While sCr did not show this high significance. Therefore, serum cystatin C has been thought to be a better marker to detect early nephropathy and early decline of GFR. This also was proved by Ref. [58]and [57], who concluded that, both cystatin C and serum creatinine were better to predict GFR decline than appearance of albuminuria. Therefore, we suggest that serum cystatin C could also reflect glomerular dysfunction. Using an increased cut-off value of 90 ml/min, the present results indicated that serum cystatin C is also a good indicator of early decline of GFR and CKD stage 1–2, compared with the previous studies [59]. [60] and [59] also found that the renal plasma clearance of cystatin C correlated well with GFR using 51Cr-EDTA. Given these characteristics, cystatin C can be considered to be an ideal indicator of GFR. The present study also found that serum cystatin C showed a good correlation to CrCl in diabetic nephropathy patients. The cause of accuracy of cystatin C is that Cystatin C is degraded in renal tubular cells and not secreted by the kidneys, which means that plasma/serum levels are dependent on GFR. As there is no other endogenous source of this protein, there is no need to measure urine levels to get a true GFR estimation unlike creatinine which need urine estimation to calculate GFR [50]. On the other hand some controversies over the accuracy of cystatin C exists. Contrary to previous reports, a large cross-sectional study suggests that factors other than renal function (age, weight, gender, smoking, and levels of c-reactive protein) may influence serum cystatin C levels

independent of GFR [34]. They found that older ages, male gender, greater weight, greater height, current cigarette smoking and serum CRP levels were independently associated with higher serum cystatin C levels after adjusting for creatinine clearance. However, cystatin C production was not thought to be affected by any factors until several authors demonstrated that serum cystatin C could be affected by both rheumatoid factor and high doses of glucocorticoids [61]. Additionally, indirect evidence including decreasing cystatin C in asthmatic patients after cyclosporine therapy [62]. Cystatin C tends to be increased in patients with hypothyroidism and decreased in those with hyperthyroidism. The production of cystatin C is differently influenced by thyroid hormone, even in mild forms. Thyroid dysfunction is increased in frequency in the diabetic population [41]. In our study, thyroid diseases patients were excluded. [34] also found that there is positive correlation between CRP and cystatin C levels in large populations indicates that inflammatory status may also contribute to changes in serum cystatin C levels. But in our study we were excluding any patients under any inflammatory conditions, so there is no controversy with this study. However many other studies proven that cystatin C level is not affected by inflammatory conditions [36] and [63]. So, the diagnostic utility of cystatin C seen in our study is similar to that previously reported by other investigators. So that most of our patients with renal dysfunction had high serum cystatin C levels at the time of s. creatinine is still near normal range. So, the evaluation demonstrates that cystatin C is a good marker for application in real time, and suggests that serum cystatin C is a better marker of GFR than serum creatinine specially in patients with type 2 diabetes mellitus.

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