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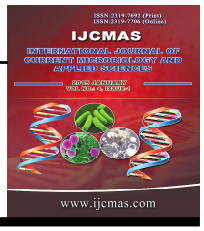

Original Research Article

A Comparative Study for Cystatin C and Some Biochemical Markers for Predicting Diabetic Nephropathy in Iraqi Patients

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Keywords

Cystatin C, Diabetic nephropathy

A B S T R A C T

Diabetic nephropathy (DN) is one of the major complications of diabetes and it is defined as a rise in the urinary albumin excretion rate and abnormal renal function. Currently, changes in albuminuria are considered a hallmark of onset or progression of DN. However, some patients with diabetes have advanced renal pathological changes and progressive kidney function decline even if urinary albumin levels are in the normal range, indicating that albuminuria is not the perfect marker for the early detection of DN. Creatinine are considered the gold standard methods for estimating GFR. Serum creatinine demonstrates an inadequate sensitivity, particularly in the early stages of renal impairment. Cystatin C, plasma protein has a low molecular mass freely filtered through the glomerulus and reabsorbed completely by tubular cells, it has been proposed as a new and very sensitive serum marker of changes in GFR. This study was designed to test whether serum cystatin C can replace serum creatinine for the early assessment of nephropathy in patients with type 2 diabetes.

Introduction

The primary microvascular complications of diabetes include damage kidneys known as diabetic nephropathy (DN), is the most common complication of diabetes (Long and Dagogo-Jack, 2011). Diabetic nephropathy is the leading cause of kidney disease in patients starting renal replacement therapy and affects 40% of type 1 and type 2 diabetic patients (Jorge *et al.,* 2005). The natural history of diabetic nephropathy is characterized by specific renal morphological and functional alterations. Features of early diabetic renal changes are microalbuminuria (30–300mg/ day), glomerular hyperfiltration, glomerular and renal hypertrophy, increased basement membrane thickness, and mesangial expansion with the accumulation of extracellular matrix proteins such as collagen, fibronectin, and laminin. Advanced diabetic nephropathy is characterized by macroalbuminuria (>300 mg/day) (Deepak and Amit, 2012). Moreover, impaired renal function may be present even in patients with normal urinary albumin excretion rate (Parchwani and

Upadhyah, 2012). The best procedures for glomerular filtration rate (GFR) measurement depend on the remove 51Cr-EDTA or iohexol, and there are impractical in clinical settings and for larger research studies.

Cystatin C (CysC) belongs to the cystatin super family of cysteine protease inhibitors. It is the reversible competitive inhibitor of C1 cysteine proteases (Ochieng and Chaudhuri, 2010). It is unique among all known cystatins that is produced at a constant rate by nucleated cells (Wagner, 2008) Cystatin C is a small 13 kD protein, which fulfills all the basic requirements for an endogenous filtration marker (Tanaka *et al.*, 2007). Cystatin C has multiple biologic functions including controlling extracellular proteolysis and modulation of the immune system. Its utility in estimating kidney function derives from the fact that after being freely filtered in the glomerulus, it is then absorbed in the kidney tubules where it is fully degraded locally (Naghavi, 2010). The production rate of cystatin C is remarkably constant over the entire lifetime and elimination from the circulation is almost completely via glomerular filtration. In the absence of significant tubular damage, cystatin C is reabsorbed and metabolized by the proximal tubular epithelial cells and is not returned to the circulation (Wagner, 2008).

Materials and Methods

A total of 44 patients were recruited from out-patient clinic of the National Centre for Diabetes Treatment and Research in Baghdad. Patients with concurrent acute illnesses, malignancy, and active immunological diseases; medical history of clinical cardiovascular disease; medical history included the diseases – hypertension, rheumatoid arthritis, anemia, bronchial asthma or medications (warfarin, acetylsalicylic acid, alpha-methyldopa, vitamins, tramadol, simvastatin) that interfered with HbA1c % measurements, and smoking history were excluded from the study. In this study selected 3 groups: (20) patients with normal-albuminuria (albumin excretion in urine persistently lower than 30 mg/d), 14 pateints micro-albuminuria (albumin excretion between 30 mg/d and 300 mg/d), and macro-albuminuria (persistent albumin excretion greater than 300 mg/d). Each group consisted of 10 men and 10 women matched for age with those of the other groups. Twenty healthy individuals matched for age and sex with the patients were included in the study as a control group. The controls were taken from relatives of diabetic patients attending the National Centre for Diabetes Treatment and Research in Baghdad who did not have any diagnosed disease or symptoms. All of the participants provided written informed consent. The anthropometric measurements and blood pressure were recorded. Biochemical testing included plasma fasting blood glucose, serum uric acid, blood urea, serum creatinin. Glycated hemoglobin HbA1c %, fasting serum lipid (triglycerides) and fasting serum lipoprotein (high density lipoprotein) were done. Serum cystatin C (Cosabio, China), insulin (Demedetec Company, Germany) were detected by enzyme-linked immunosorbent (ELISA) and relevant clinical features were collected simultaneously in all subjects.

Statistical analysis: The results were presented as sample size (n), mean ± standard deviation (SD), and standard error of mean (SEM). The statistical significance between the groups was analyzed by study t-test and correlation test between various parameters considering P-value < 0.05 as significant. All statistical significance were

done using The Statistical Analysis System-SAS (2012).

Result and Discussion

Table 1 showed the clinical characteristics of diabetic groups and control groups, there is a significant a significant difference ($P \leq 0.05$) in WHR in diabetic patients with normal-albuminurea as compared to other study groups.

Table 2 showed that there was highly significant elevation in the baseline value of Cystatin C in diabetic groups micro-albuminurea, macro-albuminurea and normal-albuminurea diabetic patients as compared with control group ($p< 0.0001$).

Serum creatinin level was found to be higher in diabetic patients groups compared with the control groups ($P < 0.0001$) as shown in table 2.

The mean serum cystatin C for diabetics nephropathy (Micro and Macro-albuminurea) (2.61 ± 0.20mg/L) which significantly higher than that both diabetic patient without completions (1.13 ± 0.11mg/L) and healthy control mean (0.810 ± 0.03mg/L) (using ANOVA test) (Table 3).

Cystatin C was more sensitive than serum criatinine in the Micro-albuminurea (76.26% and 72.08%, respactivly), Macro-albuminurea (68.95% and 61.33% respectively), Normal-albuminurea (59.62% and 55.31% respectively) while in control group serum creatinine appear more sensitive then cystatin C (47.60% and 42.64%, respectively), as shown in table 4.

The mean of insulin serum level was highly significantly increased in diabetic patients groups (Micro-albuminurea = 25.83 ± 2.04, Macro-albuminurea = 22.18 ± 1.82 and Normal-albuminurea = 17.54 ± 1.92) as compared to controls (8.58 ± 0.63 µIU/ml). There was a significant difference ($P<0.001$) in HOMA mean values between studied groups (using ANOVA test). There was a significant difference ($P<0.001$) in both plasma glucose and HOMA between diabetic patients groups and control group (Table 5).

Lipid profile of the diabetic patients groups and control are summarized in table 5. A high level of cholestrol (206.14 ± 9.07 and 218.67 ± 12.75) was observed in both DN cases (Micro-albuminurea and Macro-albuminurea, respectively) while in diabetic patients normal-albuminurae (168.93 ± 3.14) and in healthy control group (179.10 ± 2.67).

Triglyceride was found to be significantly higher ($p<0.0001$) when compare between studied groups as well as the mean of HDL was significantly difference between diabetic patient groups and in control ($P<0.05$), as shown in table 5.

The mean serum level of uric acid slightly increase in the Micro-albuminurea group (7.37 ± 0.34 mg/dl) when compared to that found in the Macro-albuminurea group (7.20 ± 0.32 mg/dl) but the difference was a significant difference ($P<0.001$) in the mean of uric acid when compared between all study groups as shown in table 7 [using ANOVA test].

Blood urea is a part of kidney function test, its levels were significantly ($p<0.0001$) higher in micro-albuminurae patients when compared with other study groups.

Diabetes has become the most common single cause of end stage renal disease (ESRD). In the U.S., diabetic nephropathy accounts for about 40% of new cases of

ESRD. Assessment and follow up of early renal dysfunction is important in diabetic nephropathy. Cystatin C concentration has been proposed as an endogenous marker of GFR superior to creatinine (Shima *et al.*, 2011). The routine classical evaluation of diabetic nephropathy includes appearance of microalbuminuria, decreased creatinine clearance and increased serum creatinine (Yun Kyung Jeon *et al.*, 2011). But, it has been reported that a decline in the renal function of patients with diabetes was not always accompanied by an increased ACR (Tsalamandris *et al.*, 1994). About 20%-30% of patients with type 2 diabetes, accompanied by renal insufficiency showed normal-albuminuria (Rigalleau *et al.*, 2007). 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 (Shima *et al.*, 2011). 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 (Uslu *et al.*, 2005). Therefore, other biomarkers for estimation of renal function have been searched for and one of them was cystatin C (Choe *et al.*, 2010). The present study confirmed that cystain C could be one of the additional tubular factors which represent kidney state of diabetic patients. In the current study, the mean serum cystatin C level showed a statistically significant increase in micro-albuminurea when compared to other groups (Table 2), such finding is in agreement with other study (Jeon *et al.*, 2011). Recent studies have mainly focused on tubular damage, which is known to correlate with acute kidney injury in patients with diabetic nephropathy (Nielsen, 2010; Nauta, 2011; Jeon, 2011; Kim, 2012; Fu, 2012; Vaidya, 2011; Macisaac, 2011). Other studies suggested that several tubular markers increase more in diabetic patients than in healthy controls, and this correlated with the severity of albuminuria (Abeer *et al.*, 2014). Serum cystatin C is the most valid marker to estimate the GFR, rather than serum creatinine, and to predict progression of renal dysfunction (Mohammad Mahdi Sagheb *et al.*, 2014).

The present study showed that cystatin C was a good marker of impaired renal function and its more sensitive marker in most study groups when compared with creatinine. Cystatin C had a good correlation with creatinine.

Table.1 Characteristics of patients

Parameters	Mean ± SD				LSD value	P-value
	Microalbuminurea	Macroalbuminurea	Normal-albuminurea	Control		
Age	58.21± 5.91	58.4±7.77	59.4±5.22	':?54.7±7.04	5.189NS	0.0943
BMI (Kg/m^2)	33.16 ± 0.43	31.78 ± 0.66	31.32 ± 0.89	31.91±0.58	1.89 NS	0.296
WHR	0.927 ± 0.01	0.920 ± 0.01	0.876 ± 0.01	0.913±0.01	0.032 *	0.020
* (P≤0.05), ** (P≤0.01), NS: Non-significant.						

Table.2 Serum Cystatin C and creatinin levels in diabetic patients groups and controls

Mean±SD (Range)	Microalbu minurea	Macroalbu minurea	normalalbu minurea	Control	LSD value	P value
Cystatin C (mg/L)	3.24 ± 0.28	2.03 ± 0.19	1.13±0.11	0.810±0.03	0.461 **	0.0001
S.Cr	2.87 ± 0.23	2.00 ± 0.12	0.873±0.02	0.815 ±0.02	0.353 **	0.0001

Table.3 The levels of cystatin C and S.Cr in diabetic patients with nephropathy complication, diabetic patient without any complications and healthy control group

Parameters	Mean ± SD			LSD value	P-value
	Diabetic nephropathy	Diabetic patients	Control		
S.Cr (mg/dl)	2.42 ± 0.15	0.873 ± 0.02	0.815 ± 0.02	0.352 **	0.0001
Cystatin C (mg/l)	2.61 ± 0.20	1.13 ± 0.11	0.810 ± 0.03	0.485**	0.0001
* (P≤0.05), ** (P≤0.01), NS: Non-significant.					

Table.4 The sensitivity of Cystatin C in all study groups

Group	Sensitivity of Cystatin C	Sensitivity of S.Cr.
Micro-albuminurea	76.26	72.08
Macro-albuminurea	68.95	61.33
Normal-albuminurea	59.62	55.31
Control (healthy group)	42.64	47.60
LSD value	9.166 **	8.345 **
P-value	0.0139	0.0144
** (P≤0.01).		

Table.5 Serum Insulin, Hba1c, FPG and HOMA in diabetic patients groups and controls

Parameters	Mean ± SD				LSD value	P-value
	Micro	Macro	Normal	Control		
Insulin	25.83 ± 2.04	22.18 ± 1.82	17.54 ± 1.92	8.58 ± 0.63	4.509 **	0.0001
Hba1c	9.49 ± 0.36	8.97 ± 0.23	8.55 ± 0.24	5.24 ± 0.14	0.697 **	0.0001
FPG (mg/di)	160.50 ± 5.78	137.73 ± 7.40	121.40 ± 5.40	85.40 ± 1.59	14.48 **	0.0001
HOMA	182.59 ± 13.76	133.53 ± 11.05	92.65 ± 8.35	32.45 ± 2.46	17.539 **	0.0001
* (P≤0.05), ** (P≤0.01), NS: Non-0significant.						

Table.6 Lipid profile in diabetic groups and control group

Parameters	Mean ± SD				LSD value	P-value
	Micro	Macro	Normal	Control		
Cholesterol	206.14 ± 9.07	218.67 ± 12.75	168.93 ± 3.14	179.10 ± 2.67	21.41 **	0.0001
TG	188.50 ± 11.89	181.67 ± 10.11	171.60 ± 2.81	159.90 ± 2.76	20.96 *	0.0354
HDL	46.07 ± 1.86	53.07 ± 2.33	53.80 ± 1.83	56.05 ± 1.51	5.33 **	0.0031
* (P≤0.05), ** (P≤0.01), NS: Non-0significant.						

Table.7 Serum uric acid and blood urea in diabetic groups and control group

Parameters	Mean ± SD				LSD value	P-value
	Micro	Macro	Normal	Control		
Uric acid (mg/dl)	7.37 ± 0.34	7.20 ± 0.32	6.06 ± 0.18	5.52 ± 0.19	0.736 **	0.0001
B. urea (mg/dl)	93.07 ± 6.77	77.46 ± 3.49	45.80 ± 2.01	39.60 ± 0.87	10.123 **	0.0001
* (P≤0.05), ** (P≤0.01), NS: Non-0significant.						

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