Assessment of Renal Function in Type 2 Diabetic Patients by Measurement of Serum Cystatin C in Al- Hilla City

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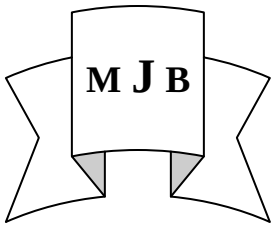

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Abstract

This study was intended to evaluate the performance of serum cystatin C and its equations for the estimation of glomerular filteration rate in comparison with serum creatinine and its equations . A total of 62 diabetic patients and 21 apparently healthy subjects as a control group were assessed. The tests done were; urine analysis , serum cystatin C , serum creatinine , HbA_1c , ,and serum glucose. The weight , height and blood pressure were measured for all subjects .

The results of this study showed a significant differences in the serum cystatin C level of the patients group (1.11±0.25mg/l , $p < 0.01$) as compared with its level in the controls group (0.76±0.08mg/l, $P<0.01$). Similarly there were a significant differences in the serum levels of glucose (224±61.9 mg/dl) , , HbA1c (9.6±2.1%) and creatinine (1.01±0.31mg/dl)($p< 0.01$)of the patients group as compared with that of controls group: (106.57 ±19.17mg/dl), (6.13%±2.7%), (0.76±0.66mg/dl) ($p<0.01$)respectively. There were a very strong negative correlations in the patients group between serum cystatin C and estimated GFRcyst+creat ($r= -0.929$, $p< 0.01$) , while the results for serum creatinine were :($r=-0.798$ $p<0.01$) .

The results of this study showed superiority of serum cystatin C and its equations over serum creatinine and its equations in detecting early decline in GFR.

Key Words: Diabetes Mellitus (D.M), Glomerular Filteration Rate (GFR), Cystatin C, Creatinine

الخلاصة

أجريت هذه الدراسة في العيادة الشعبية التخصصية في مركز مدينة الحلة على اثنان وستون مصابا بمرض السكري من النوع الثاني وواحدا وعشرون شخصا اصحاء ظاهريا كمجموعة سيطرة وكان الهدف منها هو تقييم عمل الكلى عند مرضى السكري من النوع الثاني باستخدام مادة السيستاتين سي ومقارنته بمادة الكيرياتنين المعمول بها حاليا.

بالمقارنة مع مجموعة السيطرة ، اظهرت نتائج مجموعة مرضى السكري زيادة معنوية عالية في تركيز مادة السيستاتين سي ، مادة الكرياتنين, نسبةالهيموكلوبين المتحد مع الكلوكوز ومادة الكلوكوز في الدم ($P<0.01$) . كما اظهرت ان العلاقة بين السيستاتين سي ونسبة الترشيح في الكلى ($P<0.01$ $r= - 0.929$) هي أقوى من تلك العلاقة بين الكرياتنين ونسبة الترشيح في الكلى($P<0.01$ $r=-0.798$) في مجموعة المرضى.

أظهرت نتائج هذه الدراسة تفوق مادة السيستاتين سي على مادة الكيرياتنين في اكتشاف هبوط عمل الكلى الناتج عن مرض السكري من النوع الثاني خصوصا في المراحل الأولى حينما يكون العلاج مهم جدا لمنع المضاعفات.

Introduction

Type 2 diabetes mellitus is characterized by high blood glucose level caused by relative insulin deficiency and or insulin resistance which leads to disturbance in carbohydrate, protein, fat, water and electrolyte homeostasis resulting in diabetic complications which affect the eye, kidney, nerves, skin, cardiovascular system and brain [1,2].

The GFR estimation currently depends on serum creatinine level and its equations [3]. Serum creatinine-based GFRs have many limitations; It depend on the muscle mass of the body, age, and race. It is also affected by metabolic factors as bilirubin and ketone bodies, many drugs as cimetidine and cephlosporins [4] . So there is a real need for methods that improve the detection and classification of the chronic kidney disease (CKD). One of these methods is serum cystatin C, an alternative biomarker of kidney function that is not affected by age, race, or muscle mass, freely filtered not reabsorbed and catabolized completely by the renal tubular cells [5].

This study was designed to evaluate the sensitivity of serum cystatin C level and its equations in comparison with serum creatinine and its equations.

Materials and Methods

Subjects

The patients group

The diabetic group was composed from 37 females and 25 males ,aged 25 70 years with mean ±SD(53.85±10.5) year. A full medical history about their conditions was taken and any case with urinary tract infection ,thyroid dysfunction, glucocorticoid drugs and alcohol consumption were excluded. Patients with albuminurea were carefully examined to rule out any non diabetic causes. Kidney function was assessed by measuring: serum creatinine and serum cystatin C.

The glycaemic status was assessed by measuring random blood sugar and HbA1c. BMI was calculated by dividing the weight (in kilogram)by height (in meter^2) [6].

The control group

The control group was composed of 21 apparently healthy individuals aged 30-68 years with mean ± SD (50.33±8.93) years and comprising 11 women and 10 men . The control group persons were collected from relatives and professionals working inside the clinic .

Each person in the control group had undergone a full history and physical examination and the biochemical tests mentioned above were done for the control also. Smokers , hypertensives and alcohol drinkers were excluded .

Methods

Serum glucose was measured by the commercial kit using glucose oxidase method using a kit supplied by RANDOX Laboratories (U.K). Glyaecated haemoglobin was estimated by affinity chromatography provided by Spectrum company (Egypt). Serum concentration of cystatin C was determined by enzyme linked flourescent immuno assay (ELFA) technique using TOSOH system analyzer to perform all sample and reagent operations automatically supplied by TOSOH corporation (Japan). For determination of creatinine in serum and urine the routine Jaffe method was used supplied by BIOLABO (France). Serum urea was determind by the modified urease –Berthelot method provided by Biomaghreb (Tunisia).

The equations used for estimating the glomerular filteration rate were :
1.for cystatin C alone : eGFR = by 76.7 × (cystatin C in mg/L)−1.19[7].
2.for creatinine alone (MDRD) : eGFR = 175× serum creatinine - 1.154 × age - 0.203 × 0.742 (if female) [7].
3.for both : eGFRcyst.+creat.= 177.6 × (serum creatinine in mg/dl)−0.65× (cystatin C in mg/L)−0.57×(age in years)−0.20× (0.82 if female)[8]. eGFR is reported in ml/min per 1.73 m^2 body surface area.

Results

Age:

The results of this study showed no significant differences in age between diabetic and control groups.

Table 1 Mean and standard deviation of age in diabetic and control groups.

Parameter	Subjects	No.	Mean ±SD	P-value
Age (Years)	Control group	21	50.33±8.93	**p>0.05**
	Diabetic group	62	53.85 ±10.5	

Gender:

From the 62 diabetic patients there were 35 females and 27males representing 56.45% and 43.55% respectively while the percentage of both sexes was equal in the control group .

Body mass index:

The results of this study showed a significant differences ($p<0.01$) in the body mass index between the control group and the diabetic groups .

Table 2 Mean ±Standard deviation (SD) of BMI in control and patient groups.

Parameter	Control	Patient	P value
BMI Mean±SD	34.58 ±11.93	28.93 ±7.25	<0.01

Measured parameters in the control and patient groups:

The mean± SD of the measured parameters in the control and patient groups were shown in table(3).

Table 3 The mean± SD of the measured parameters in the control and patient groups

Parameter	Control: Mean ± SD	Patient : Mean ± SD	P value
Cystatin C mg/l	0.65±0.08	1.11±0.25	P<0.01
S. Creatinine mg/dl	0.78±0.26	1.01±0.31	P<0.01
S. Glucose mg/dl	106.57±19.17	224±61.9	P<0.01
HbA1c %	6.13%± 2.7%	9.6%±2.1%	P<0.05
eGFR cyst + creat ml/min/1.73m^2	112.98±15.2	68.8±18.1	P<0.01
eGFRcreat.(MDRD) ml/min/1.73m^2	102.01±15.31	57.9±15.2	P<0.01
eGFRcyst ml/min/1.73m^2	107±15.45	68.83±16.6	P<0.01

These results showed a significant differences between the values of measured paramters in the control and the patients groups (p<0.01). It also showed that the mean and ±SD of serum levels of cystatin C in diabetic patients differ by 1.7 times than that of control group while serum creatinine levels in diabetic group differ by 1.2 times only.

Correlations between measured parameters in patients group:

The correlations between measured parameters were shown in table(2).

Table 4 Correlations between measured parameters in patients group

Parameter	S.Cystatin C	S. Creatinine
S.cystatin C mg/dl	-	r=0.873 p<0.01
S.creatinine mg/dl	r=0.873 p<0.01	-
S.glucose mg/ml	r=0.287 p<0.05	r=0.337 p<0.01
HbA1c%	r=0.343 p< 0.01	r=0.384 p<0.01
e GFRcyst.+creat. ml/min/1.73m2	r=-0.922 p<0.01	r=-0.763 p<0.01
e GFRcyst . ml/min/1.73m2.	r=-0.984 p<0.01	r=-0.783 p<0.01
eGFRcreat . MDRD ml/min/1.73m2	r=-0.892 p<0.01	r=-0.813 p<0.01

These results showed that the correlations between all parameters are significant (p<0.01 and <0.05) but there were :

1.Stronger negative correlation between eGFRcyst + creat and s.cystatin C (r=-0.922) than that with S.creatinine(r=-0.763) .
2.Stronger negative correlation between eGFRcreat. and s.cystatin C (r=-0.892) than that with S.creatinine (r= -0.813).
3.Stronger negative correlation between eGFRcyst.and s.cystatin C (r= -0.984) than that with s. creatinine (r= -0.783) .

Duration of diabetes mellitus and markers of renal function in patients group :

The duration of the diabetes mellitus was subdivided into three sub- groups:
A.from 6months-5years.
B.from6-10 years.
C.from 11-20 years.

There was progressive decline of eGFRcyst+creat from 71.65 ml/ min/ 1.73m^2 in the first sub-group to 54.82 ml/min/1.73m^2 in the third sub-group .

The correlations of eGFRcyst+creat > 60ml/min/1.73m^2 with S.cystatin C and S.creatinine in patients group:

There was a stronger negative correlation between eGFR > 60 ml/min/1.73m^2 and S.cystatin C (r= -0.867 p <0.01) , than that with S. creatinine (r = - 0.586 p<0.01) as shown in the figures (1,2) .

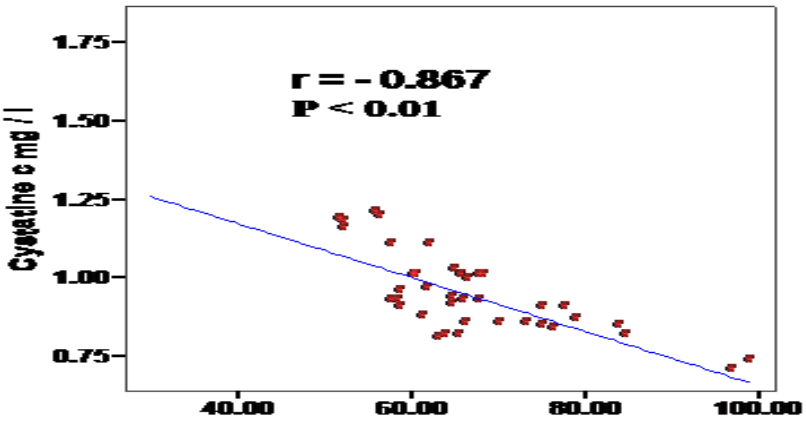

Figure 1 eGFR > 60 ml/min/1.73m^2 and s.cystatin C

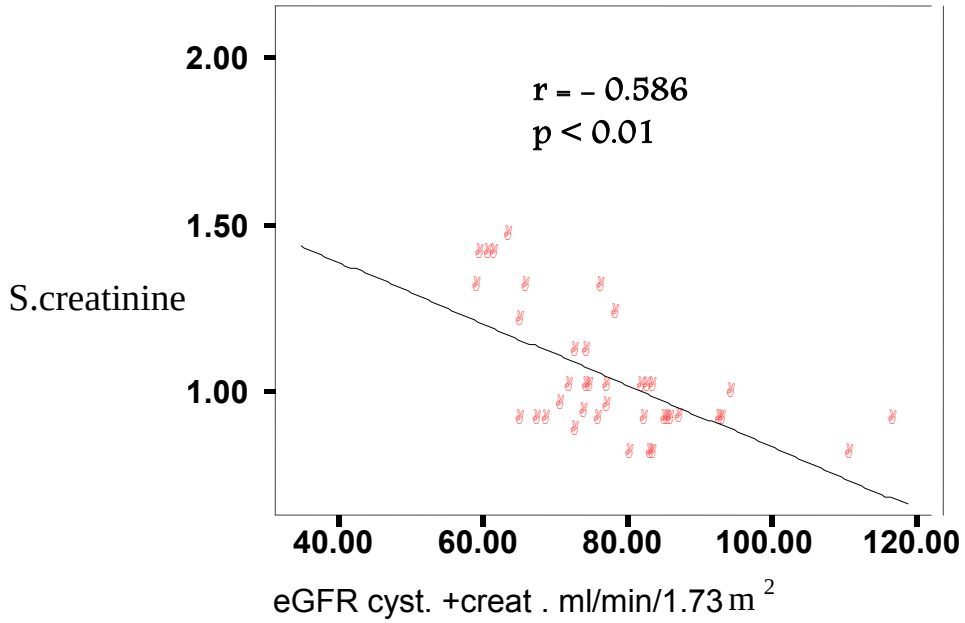

Figure 2 eGFR cyst+creat > 60ml/min/1.73m2 and S.creatinine

Correlations of eGFR cystatin C alone with some of the measured parameters in patients group:

From this study it was noticed that there is a significant positive correlation between eGFRcyst C (alone) with : eGFRcyst+crea t(r=0.984 p<0.01), eGFRcreat (MDRD) (r=0.947 p<0.01). Also it was noticed that there is a significant negative correlation between eGFRcyst.(alone) and S.creatinine (r= -0.783 p<0.01) .

Sensitivity of the tests :

Table (3) showed that the sensitivity of S. cystatin C was 90.7% and for S. creatinine it was 70% when the cut-off values were taken at 0.8 mg/l and 1.3mg/dl respectively.

Table 5 The sensitivity of S. cystatin C and S. creatinine

Parameter	Cut-off value	Sensitivity
S.Cystatin C	0.8 mg/l	90.7 %
S.Creatinine	1.3 mg/dl	% 70

Discussion

BMI represents a relative measure of body adiposity. Diabetic patients lose weight as the disease progresses due to insulin deficiency and or insulin resistance which causes increased lipolysis and proteolysis by the actions of catechoamines and other counteract hormones[9].

The results in Fig.(1) showed a stronger negative correlation between eGFR>60ml/min/1.73m2 and s. cystatin C (r = - 0.867 p<0.01) than that with s. creatinine (r= - 0.586 p<0.01) and this might be due to the blind area of the creatinine . The blind area of creatinine in turn is due to the increased secretion of this substance from the renal tubules

as the serum level of creatinine starts to elevate till it reaches to a level when the capacity of its secretion is saturated while the cystatin C is not secreted but completely catabolized by the renal tubules [10]. This result agreed with Stevens L, Coresh J, Feldman H, et al. study [11].

For diabetic patients, Pucci et al. [12] observed a higher predictive value in the early detection of reduced renal function (determined by the iohexal plasma clearance method) when the GFR was estimated by cystatin C compared with GFR estimation by the creatinine-based MDRD and Cockroft-Gault formulae. Particularly poor precision and accuracy of creatinine-based eGFR equations with respect to the range of 60–89 mL/min/1.73 m2 (determined by the isotopic ^{51}creatinine EDTA method) have also been described by others[13].

Peralta and co-workers found that cystatin C can be used to confirm a high risk groups whose estimated GFR is decreased[14,15].

The derterioration in renal function as the duration of the disease was increasing(eGFR was decreasing from 71.65 ml/min/1.73m^{2}in the first sub-group to 54.82 ml/min/1.73m^2 in the third sub-group) might occure due to the thickening of the glomerular basement membrane (GMB) caused by the activation of polyol pathway and formation of advanced glycated end products (AGEs) by the sustaind hyperglycaemia resulting in abnormalities in GMB function. The mechanism of this process is that when there is increasing general vascular permeability ,the plasma proteins reach the peritubular fluid where they are endocytosed and then degraded by lysosomes into the constituent amino acids. Proximal tubular cell reserve capacity for this process is limited and the increasing protein load leads to organelle congestion, lysosomal swelling and rupture, eventually exposing the cell cytoplasm and renal interstitium to the injurious effect of lysosomal enzymes. Therefore, an increase in filtered protein with advanced renal disease may accelerate the turnover rate of the lysosomal system resulting in progressive decline in renal function[16].

Several studies have suggested that s.cystatin C is a superior marker for renal function than creatinine [17].

Conclusion

Serum cystatin C and its equations are better predictors of early changes in the kidney of type 2 diabetic patients as compaired with serum creatinine and its equations

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