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Novel Renal Biomarker Cystatin C for Diagnosis of Impaired Renal Function for Uncontrolled Iraqi Diabetics

Inaam Ahmed Amin

Dept. of Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad Iraq
Inaamalchalabe@gmail.com

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ABSTRACT

Diagnosis of mild or moderate impaired renal function is very important especially for uncontrolled diabetic patients. The study was conducted in the National Diabetes Center/Al-Mustansiriya University. Renal biomarkers were used to evaluate the reduced renal function in 44 uncontrolled diabetic patients (fasting blood glucose is more than 125 mg/dl), by estimating glomerular filtration rate GFR which represents the best overall index of kidney function. These biomarkers include urea, creatinine and cystatin C as a novel renal biomarker. Elevated levels of serum urea nitrogen more than normal range with mean=39.29 and SD=+19.36 serum creatinine within the normal range with mean=0.78 and SD=+0.26. The ratio of serum urea nitrogen/serum creatinine significantly high than normal with mean=49.91 and SD=+15.42. This high ratio of urea/creatinine shows a definite renal impairment. Serum cystatin C levels also shows a high values more than the normal range with mean=1.95 and SD=+0.93. Estimating the glomerular filtration rate GFR, by the application of Modified Diet Renal Disease (MDRD) equation for serum creatinine, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for serum cystatin C and also (CKD-EPI) equation for both serum creatinine and serum cystatin C. The estimated GFR with cystatin C (by application of CKD-EPI equation) is more correlated to the estimated GFR for both serum creatinine and serum cystatin C (by application of CKD-EPI equation) with r =0.59 while the correlation of estimated GFR for serum creatinine (by application of MDRD equation) is less correlate to the estimated GFR for both serum creatinine and serum cystatin C (by application of CKD-EPI equation) with r =0.56. The study findings support that the cystatin C, as a new novel GFR marker providing more sensitivity and overcoming many limitations of the creatinine-based methods. So, serum cystatin C levels indicate the exact stage of the impaired renal function by the estimation of GFR for the uncontrolled diabetic patients.

Keywords: Cystatin C, GFR, uncontrolled diabetes, renal biomarker

1. Introduction

Cystatin C is a novel serum marker of glomerular filtration rate (GFR), a critical measure of normal kidney function. Cystatin C is produced by all nucleated cells (Burtis et al., 2006), regulated by a so-called 'housekeeping' gene, released into blood stream with a half-life of

2hr, its production rate is remarkably constant over the entire lifetime. 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, 2010). Therefore, Cystatin C serum concentration correlates closely to the glomerular clearance rate. So, it is considered as an accurate endogenous GFR marker (Grubb, 2000). In this study, serum Cystatin C levels for uncontrolled diabetic patients, those have a severe form of diabetes which is completely incurable or it is not treated adequately enough possibly due to lack of information (fasting food sugar is more than 125mg/dL), were estimated. Creatinine serum levels were estimated too, creatinine is an important biomolecule because it is a major by-product of energy usage in muscle, it is produced at a fairly constant rate by the body (depending on the muscle mass). Creatinine is chiefly filtered out of the body by the kidneys (glomerular filtration and proximal tubular secretion). If the filtering of the kidney is deficient, creatinine blood levels rise, so the measurement of serum creatinine is an important indicator of renal function (Taylor, 1989). Serum urea nitrogen was also estimated, during the metabolism of protein in the body, the liver creates ammonia, which is broken down into a by-product urea. Kidneys filter excess urea into the urine and in sweat, but some goes into the blood stream as serum urea nitrogen, serum urea nitrogen is directly related to the excretory function of the kidneys. It serves as an index for the renal function. A markedly increased urea in the blood is conclusive evidence of severe impairment of glomerular function. A more complete estimation of renal function can be made when interpreting the blood (serum) concentration of creatinine along with that of urea. Serum urea nitrogen/serum creatinine (the ratio of blood urea nitrogen to creatinine, both are filtered by the kidney and extracted in urine). This ratio can indicate other problems besides those intrinsic to the kidney, for example, the increased ratio may indicate a prerenal problem such as volume depletion. A comparison study was established for serum cystatin C versus serum creatinine as a marker of early deterioration of GFR in a patients with uncontrolled diabetes, the effect of age on the two GFR markers was evaluated to separate the effects of age from disease, by the application of modified diet renal disease (MDRD) equation for creatinine (Levey et al. 1999) and chronic kidney disease epidemiology collaboration (CKD-EPI) equation for cystatin C (Larsson et al. 2004). More accurate equation (CKI)-EPI in which both serum creatinine and serum cystatin C were developed (National Kidney Foundation, 2002).

2. Patients and Methods

The study was conducted in the National Diabetes Center/Al-Mustansiriya University, a total 44 patient; ages ranged (22-76) years, 24 males with mean age (56.00+12.40) and 20 females with mean age (52+10.72). According to the estimation of fasting blood glucose, they have an elevated level of glucose (128-405 mg/dl), they are uncontrolled diabetic patients. 21 Patients of type 1 diabetes, 11 males/10 females with age range (22-68) years and the other 23 patients of type 2 diabetes 13 males/10 females with age range (29-76) years. Enzymetic method was applied for determination of glucose (SPINREACT com.). glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H2O2) is detected by a chromogenic oxygen acceptor, phenol-aminophenazone in the

presence of peroxidase (POD). The intensity of the color formed is proportional to the glucose concentration in the sample (Kaplan et al., 1984; Trinder, 1969). One ml working reagent which is a mixture of TRIS PH7.4 with phenol and enzymes (Glucose oxidase, peroxidase with 4-Aminophenazone), is added to 10□l of sample and standard, incubate for 20 minutes at room temperature. Then, reading the absorbance of both sample and standard against the blank. For serum cystatin C, the application of the quantitative sandwich enzyme immunoassay technique (CUSABIO com.). Antibody specific for cystatin C has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any cystatin C present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for cystatin C is added to the wells. After washing, avidin conjugate horseradish peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of cystatin C bound in the initial step. The color development is stopped and the intensity of the color is measured (Lequin, 2005). Creatinine JAFFE Reaction: For the determination of serum creatinine, in an alkaline solution, creatinine reacts with picric acid to form a coloured complex. The rate of formation of the complex is measured and it is directly proportional to the amount of creatinine in the sample (Jaffe, 1886). One ml of working reagent (alkaline buffer with picric acid) is added to 10□ of both sample and standard, gently mix and incubate for 20 minutes at 25oC, measure the absorbance of sample and standard against blank. Urease Modified Berthlot Reaction: (BIOMERIEUX com.) is applied for the determination of urea concentrations. Urease hydrolyzes urea by producing ammonium. In an alkaline medium, the ammonium ions react with the salicylate and hypochlorite to form a green colored indophenol (2-dicarboxylindophenol). The reaction is catalyzed by the sodium nitroprusside. The color intensity is proportional to the urea concentration in the sample (Fawcett et al., 1960; Patton et al., 1977). One ml of the working reagent was prepared by the addition of urease enzyme to a mixture of (phosphate buffer pH8, sodium salicylate, sodium nitroprusside and EDTA), then added to 10□l of sample and standard, incubate for 5 minutes at room temperature, after that 200□l of alkaline reagent (sodium hydroxide and sodium hypochlorite) is added to the reactant and incubate for additional 10 minutes at room temperature. Then reading the absorbance of both the sample and standard.

3. Results and Statistical Analysis:

After collection and categorization of data of the 44 patients, statistical analysis was conducted by using unpaired t-test and coefficient of correlation was estimated also. All analyses were submitted by SAS program.

A) The biochemical parameters for the 44 patients, were established in table 1, shows the mean, standard deviation, minimum and maximum values for these parameters. In comparison with normal values that listed by the National Diabetes Center, except cystatin C normal values from other references.

Table (1)

Biochemical parameters	Mean	SD	Min	Max	Normal values
Fasting blood glucose	211.25	±70.61	128	405	75-109mg/dl
Serum cystatin C	1.95	± 0.93	0.75	4.4	0.52-0.98mg/dl
Serum urea	39.29	±19.36	20	112	20-45mg/dl
Serum creatinine	0.78	±0.26	0.5	1.5	0.7-1.3mg/dl
Urea/creatinine ratio	49.91	±15.42	24.57	80.55	10:1-20:1

B) Glomerular filtration rate (GFR) was estimated by modified diet renal disease (MDRD) equation for creatinine. And chronic kidney disease epidemiology collaboration (CKD-EPI) equation for cystatin C. In addition to the more accurate (CKD-EPI) equation in which both serum creatinine and serum cystatin C were developed. The estimated GFR by the 3 mentioned equations shown in table 2.

Table (2)

Estimated GFR	Mean	SD	Min	Max
1-Modified Diet Renal Disease (MDRD) for creatinine	86.14	±25.80	21.49	121.28
2-Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) for cystatin C	50.52	±20.33	15.78	97.11
3-(CKD-EPI) equation for both creatinine and cystatin C	66.81	±20.19	35.45	121.64

LSD = 9.3922

All the applied equations were adjusted for age and sex. The means of the estimated GFR of the three equations differ significantly ($P<0.05$). The correlation between equation (1) and (2) equal to $r = 0.34$ with $P= 0.02$ (significant). The correlation between equation (1) and (3) equal to $r=0.56$ with $P=0.0001$ (significant). The correlation between equation (2) and (3) equal to $r=0.59$ with $P=0.0001$ (significant). The Kidney Disease Improving Global

Outcomes (KDIGO) in 2004 issued the first international guidelines on CKD, including a definition and classification of (KD), as shown in table 3.

Table (3)

Stage	GFR (ml/min/1.73m^2)	Description
1	≥90	Normal or elevated GFR
2	60-89	Mild GFR reduction
3	30-59	Moderate GFR reduction
4	15-29	Severe GFR reduction
5	< 15	Renal failure

GFR = glomerular filtration rate

GFR determination provides the basis for detection and classification of CKD. The GFR usually expressed in ml/minute/1.73m2 and provides the volume of blood that is cleared per minute by the kidneys, standardized for the body surface, which is 1.73m2 for the average person. According to the (KDIGO) staging which depends on GFR, the estimated GFR of the patients included in the study is categorized in table 4.

Table (4)

No.	eGER	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
1-	MDRD for creatinine	52.27% 23 patients	34.09% 15 patients	11.36% 5 patients	2.2% 1 patient	-
2-	CKD-EPI for cystatin C	4.54% 2 patients	27.27% 12 patients	56.81% 25 patients	11.3% 5 patients	-
3-	CKD-EPI for both creatinine-cystatin C	13.63% 6 patients	50% 22 patients	34.09% 15 patients	2.27% 1 patient	-

eGFR = estimated GFR , MDRD = Modified Diet Renal Disease , CKD-EPI=Chronic Kidney Disease Epidemiology Collaboration

4. Discussion

Uncontrolled diabetes is a severe form of diabetes which is completely incurable or it is not treated adequately enough possibly due to lack of information. A high level of blood sugar (100mg/dl and more) can affect nearly every organ in the body including heart, kidneys, eyes, nerves, gums and teeth. Persisting of a high level of blood sugar causes serious

medical complications, because the kidneys become overloaded and the heart and lungs get stressed. Increased blood flow through nerves damages the blood vessels. Patients with uncontrolled diabetes can develop vision problems as a result of injuries to the retina or optic nerve. They may also develop seizures, can relapse in to coma, and eventually die. The initial stage of diabetes leads to uncontrolled diabetes when ignored. Early treatment for any type of complication or to keep the blood sugar levels within the safe range can prevent from serious damage. Only minor lifestyle changes are necessary to prevent the dreaded consequences of uncontrolled diabetes. Diabetic nephropathy is one of the most important disorder that should be followed (Perkins et al., 2005), and the importance of detecting early impairment of renal function in patients with diabetes is evident (Ritz, 1999). The sooner kidney dysfunction is diagnosed and treated the greater odds of preserving remaining nephrons and preventing the need for dialysis. Different renal biomarkers were used for the diagnosis of kidney function. Blood urea is directly related to the excretory function of the kidneys, it serves as an index for its function. A markedly increased blood urea is conclusive evidence of severe impairment of glomerular function. Two factors interferes with blood urea, protein intake and hydration level. Creatinine is most widely used biomarker of kidney function, it is inaccurate at detecting mild renal function, and levels can vary with muscle mass and protein intake. Formulas for estimating GFR which represent the best overall index of kidneys function (National Kidney Foundation, 2002), such as the Cockcroft and Gault formula, the modification of diet in renal disease (MDRD) formula try to adjust for these variables (Wagner, 2010). Creatinine is more specific and sensitive indicator of kidney disease than blood urea, although in chronic renal disease, both blood urea problems. Serum urea to serum creatinine (the ratio of serum urea to creatinine) can indicate other problems besides those intrinsic to the kidney, for example the increased ratio may indicate a pre-renal problem such as volume depletion. Cystatin C, a new GFR marker providing more sensitivity and overcoming many limitations of the creatinine-based methods. A critical measure of normal kidney function. Serum levels of cystatin C are more precise test of kidney function (as represented by GFR) than serum creatinine levels (Roos et al., 2007). Serum cystatin C levels started to increase to greater than normal values when GFR was 88ml/min/1.73m2, whereas serum creatinine level began to increase to greater than normal when GFR was 75 ml/min/1.73m2, these data suggest that measurement of cystatin C may be useful to estimate GFR, especially to detect mild reductions in GFR, and therefore may be important in the detection of early renal insufficiency in a variety of renal diseases for which early treatment is critical (Dharnidharka et al., 2002). Cystatin C may be used as an alternative to creatinine and creatinine clearance to screen for and monitor kidney dysfunction in those with known or suspected kidney diseases. It may be especially useful in those cases where creatinine is not appropriate, for instance, in those who have liver cirrhosis, are very obese, are malnourished or have a reduced muscle mass. Measuring cystatin C may also be useful in the early detection of kidney disease, when other test results may still be normal and an affected person may have few, if any, symptoms (Elisabeth et al., 2000). Despite this cystatin C has had only limited commercial success to replace creatinine as an indicator of GFR, perhaps due to the greater cost of measurement (American Association for Clinical Chemistry, 2012). Cystatin C has an essential requirement to be a novel GFR marker as its elimination exclusively via renal filtration, while creatinine can be alternatively secreted via the tubulus system. This

alternative elimination pathway compensates for a decrease in GFR and keeps the serum creatinine level unchanged until GRF has declined to 60ml/min/1.73m2. Creatinine levels only increase if the capacity of the alternate tubular secretion pathway is fully used; this is why there is a 'creatinine blind range' considerably limiting the sensitivity and precision of creatinine in the normal and slightly reduced GFR range. In addition, with cystatin C, a constant relationship between analyte and GFR is observed, the decline of renal function with aging is reflected by increasing cystatin C levels in the elderly in a sensitive manner. Cystatin C levels reach adult levels from one year of age, whereas creatinine levels increase as long as the muscle mass is growing in children and teenagers (Chew et al., 2008). Thus, a unique reference range can be applied for cystatin C for males and females from one year of age, whereas the interpretation of creatinine requires age and sex specific reference ranges (Wagner, 2010). Moreover, a sensitive GFR determination of cystatin C with one serum or plasma sample. For the conducted study, application for eGFR by different equations (MDRD for serum creatinine, CKD-EPI for both serum cystatin C and serum creatinine with serum cystatin C), shows that is creatinine less sensitive for the decline of GFR and the concentration of serum creatinine is elevated when there is a severe renal dysfunction. While cystatin C show more reliable results than do creatinine, its serum concentration elevated significantly with mild and moderate of 37 patients (84.08%) if compared to that of creatinine which include 20 patients (45.45%), these values indicate the presence of kidney disease and represent an increased risk of impaired kidney function, progression to kidney failure, and premature death caused by cardiovascular events of patients with kidney disease (Finney et al., 2000; Hock et al., 2003). Finally, cystatin C determination could be a valuable tool for uncontrolled diabetic patients, for early diagnosis of mild or moderate impaired renal function.

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