ORIGINAL ARTICLE

Cystatin C: A Better Predictor of Kidney Function in Diabetic Patients

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ABSTRACT

Serum cystatin C is a new promising marker of renal function. The aim of this study was to analyze serum cystatin C as a better predictor of renal function in diabetic nephropathy. In 60 diagnosed diabetic patients, serum cystatin C and serum creatinine were assessed. Glomerular filtration rate was estimated based on the cystatin C concentration according to Cockcroft- Gault formula and based on serum creatinine concentration according to Larsson formula. DTPA-GFR (Diethylenetriamene pentaacetate Renogram) was done as reference standard. The cross tabulation of DTPA-GFR was done with eGFR- creatinine and eGFR-cystatin C. The calculated sensitivity, specificity and accuracy of eGFR- creatinine were 85%, 87.2% and 85% respectively. The eGFR- cystatin C showed higher sensitivity, specificity and accuracy than eGFR- creatinine in studied diabetic subjects. The cystatin C showed more significant correlation, $r = 0.78$, $p < 0.001$ than serum creatinine, $r = 0.59$, $p < 0.001$ with DTPA-GFR in diabetic patients. This study demonstrates that serum cystatin C may be used for early prediction for renal function impairment in diabetic kidney disease.

Key Words: Cystatin C, DTPA-GFR, eGFR-creatinine, eGFR-Cystatin C

Abreviation: eGFR- Estimated Glomerular Filtration Rate

Introduction

Diabetes mellitus is a metabolic disease characterized by defects in insulin secretion, insulin action or both. The number of people with diabetes is increasing due to population growth, aging, urbanization and the increasing prevalence of obesity and physical inactivity. Approximately 40% of patients with type I diabetes and 5-15% of patients with type II diabetes eventually develop End Stage Renal Disease (ESRD)[1]. Even a diabetic patient is under treatment, there is a risk of development of nephropathy. The risk is related to the length of time the person has diabetes. There is good evidence that early treatment delays or prevents the onset of diabetic nephropathy. Therefore prevention of diabetic renal disease or at least the postponement of or slowing down the disease process, has emerged as a key issue[2]. However our ability to assess renal function is poor in early diabetic nephropathy, when active management is important[3]. Glomerular filtration rate is the best overall index of renal function in health and disease. While insulin, 51cr- labeled EDTA, 99mTc- labeled DTPA plasma clearance are considered as the gold standard methods for estimating glomerular filtration rate.

Serum creatinine and creatinine clearance are the most widely used indices for the routine noninvasive estimation of GFR. Creatinine is usually measured by the Jaffe reaction, based on a complex formation between alkaline picrate and creatinine[4].The serum creatinine concentration may be significantly influenced by

several extra renal factors. Serum creatinine is considered relatively specific but not very sensitive because serum creatinine remains in the normal range until 50% of renal function is lost. This is creatinine blind area. Here GFR range is between 40 -90ml/min/1.73m^2. It is the area where mild to moderate decrease in GFR occurs. An early reduction of GFR does not show with serum creatinine in the creatinine blind area. Serum creatinine will give false negative results in creatinine blind area. Various creatinine based equation have been developed in an attempt to improve the estimation of GFR from serum creatinine. Among these, the Cockcroft- Gault (C&G) formula and the modification of diet in renal disease (MDRD) formula are widely used. But those are also limited by lack of validation in the full range of GFR to which they are applied[4]. So a more precise and accurate marker of GFR as an assessment of renal function would be clinically useful.

Cystatin C has been proposed as a good marker of GFR, particularly in patients with mild to moderate renal impairment[5]. It is a nonglycosylated protein belonging to the cystene super family (inhibitors of cystene proteinases). Cystatin C is produced by all nucleated cells and its rate of production is constant. The production is not altered by inflammatory conditions, is not related to lean muscle mass. Because of its low molecular mass (approx. 13000 kpa) and its positive charge at physiological PH, cystatin C easily crosses the glomerular filter, after filtration, the proximal tubular cells reabsorb and catabolize virtually all of the filtered cystatin C. It was demonstrated that the renal clearance of cystatin C is closely related to GFR, measured as 51-CR-EDTA clearance. Cystatin C does not have a blind area will therefore show a positive reaction of GFR. One of most significant advantages of cystatin C in comparison with traditional markers of renal impairment is that very small reductions in GFR cause significant increase in cystatin C serum levels[2].

From pathophysiological point of view earlier the diagnosis better will be the prevention of progress in diabetic nephropathy. So it is essential to detect the early impairment of renal function in diabetic patient. This study is aimed to assess the performance of serum cystatin C as an early marker of GFR in diabetic nephropathy and to take preventive measures.

Materials And Methods

A cross-sectional study was conducted in the outpatient department of BIRDEM hospital during the period from July 2008 to June 2009. A total 60 diagnosed patients of both sexes were selected as cases. Ethical clearance for the study was taken from the Ethical Review Committee of DMC and BIRDEM hospital. Exclusion criteria were history of diabetes mellitus for more than 5 years, rheumatic diseases, malignancy, cardiac diseases and drug history of taking steroids or cyclosporine.

After maintenance of all aseptic precautions, 5 ml of venous blood was drawn from each subject and transferred to a clean, dry test tube to clot. Then the sample was centrifuged and serum was collected in an eppendorf tube and preserved at -20 ^{0}C. Then serum creatinine and serum cystatin C were measured. Estimated GFR were calculated from Cockcroft-Gault formula and Larson formula respectively. DTPA-GFR was done as reference standard. Finally comparison was done between two diagnostic procedures, eGFR- creatinine and eGFR-cystatin C with reference standard (DTPA- GFR).

Serum cystatin C concentration and serum creatinine concentration were estimated by particle-enhanced Immunonephelometry using the BN Systems[6] and Jaffey reaction method[7] respectively. Finally DTPA renogram was done (Peace Harbor Hospital Imaging Services, 2005).

Statistical Analysis: All data were recorded and expressed as mean ± standard deviation (SD). Statistical analysis was performed by using SPSS software package program. Sensitivity, specificity and accuracy were measured for

eGFR- Cystatin C and eGFR-creatine. The ROC (Receiver Operator Characteristics) curve was constructed. The Pearson correlation analyses were performed for eGFR-Cystatin C and eGFR-Creatinine with DTPA-GFR. Agreement test was done between two diagnostic procedures.

Results

Table 1 shows the descriptive statistics of the outcome variables. Mean (±SD) Serum Creatinine of the subjects was 0.83(±0.25) mg/dl, Mean (±SD) Serum Cystatiin C was 0.94(±0.09) mg/L, Mean (±SD) eGFR-creatinine was 109.10(±15.51) ml/min/m2, Mean (±SD) eGFR-cystatinC was 96.75(±10.91) ml/min/m2.

Table I: Descriptive statistics of the outcome variables

Variables	Number	Minimum	Maximum	Mean	Std.Deviation
Serum Creatinine	60	.48	1.70	0.83	0.25
Serum Cystain C	60	.68	1.14	0.94	0.09
eGFR -Creatinine	60	69.38	154.21	109.10	15.51
eGFR -CystatinC	60	67.71	129.25	96.75	10.91

Table 2 shows cross tabulation of serum Creatinine versus serum Cystatin C. Out of 16 subjects, who had abnormal serum creatinine, 3 had normal serum Cystatine C. Out of 44 subjects, who had normal serum Creatinine 3 had abnormal serum Cystatin C. The agreement test done between two diagnostic tests, serum cystatine C and serum creatinine. The agreement test showed K = 0.74 that means good agreement. The cut off value of serum creatinine is 1.2 mg/dl and serum Cystatin C is 0.98 mg/L.

Table II: Serum Creatinine versus Serum Cystatin C cross tabulation

Serum Creatinine	Serum Cystatin C		
Abnormal		Normal	Total
Abnormal	13	3	16
Normal	3	41	44
Total	16	44	60

Table III: eGFR- Creatinine versus eGFR-DTPA cross tabulation

Serum Creatinine	Serum Cystatin C	
Abnormal	Normal	Total
Abnormal 10	6	16
Normal 3	41	44
Total 13	47	60

Sensitivity = 85%; Specificity = 87.2%; Accuracy = 85%

Table 3 shows cross tabulation of eGFR-Creatinine versus eGFR DTPA. Out of 16 subjects, who had abnormal eGFR-creatinine, 6 had normal eGFR DTPA .Out of 44 subjects,who had normal eGFR-Creatinine 3 had abnormal eGFR DTPA . Sensitivity, Specificity and Accuracy of eGFR-Creatinine was 85%, 87.2% and 85% respectively.The cut off value of normal GFR is 90 ml/min/m^2.

Table IV: eGFR- Cystatine C versus eGFR-DTPA cross tabulation

Serum Creatinine C	Serum Cystatin C	
Abnormal	Normal	Total
Abnormal 12	4	16
Normal 1	43	44
Total 13	47	60

Sensitivity = 92.3%%; Specificity = 91.5%; Accuracy = 98.33%

Table IV shows cross tabulation of eGFR-Cystatine C versus eGFR-DTPA. Out of 16 subjects, who had abnormal eGFR-Cystatine C, 4 had normal eGFR-DTPA .Out of 44 subjects, who had normal eGFR-Cystatin C 1 had abnormal eGFR DTPA. Sensitivity, Specificity and Accuracy of eGFR-Cystatine C was 92.3%, 91.5% and 98.33% respectively. The cut off value of normal GFR is 90 ml/min/m^2.

Table V: Correlation test between eGFR-Creatinine and eGFR-DTPA

Serum Creatinine C	Serum Cystatin C	
Abnormal	Normal	Total
Abnormal 10	6	16
Normal 3	41	44
Total 13	47	60

Pearson's r = 0.59; p < 0.001

Table V shows correlation between eGFR-Creatinine and eGFR DTPA. Statistical analysis with correlation test showed significant result. Here r = 0.59, p < 0.001.The cut off value of normal GFR is 90 ml/min/m2.

Table VI: Correlation test between eGFR- Cystatine C and eGFR-DTPA

Serum Creatinine C	Serum Cystatin C	
Abnormal	Normal	Total
Abnormal 12	4	16
Normal 1	43	44
Total	13	47 60

Pearson's r = 0.78; p < 0.001

Table VI shows correlation between eGFR-Cystatine C and eGFR DTPA. Statistical analysis with correlation test showed significant result. Here r = 0.78, p < 0.001.The cut off value of normal GFR is 90 ml/min/m2

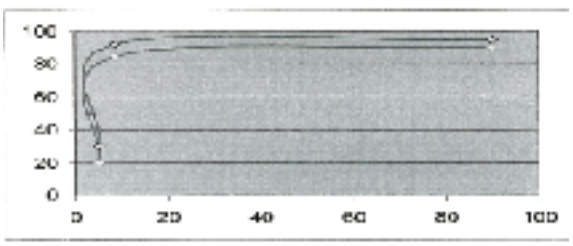

Figure I: The ROC (Receiver Operator Characteristic) Curve plots to assess the diagnostic efficiency of serum Cystatin C & serum Creatinine estimated GFR. The area under curve (AUC) of eGFR-Cystatin C is 0.88 and eGFR-Creatinine is 0.85. So, the accuracy of the tests are good.

Discussion

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[8].

In this cross sectional study, serum Cystatin C, serum creatinine & serum glucose has been measured. The age of the study subjects ranged from 32-60 years; mean ± SD of age were 40.67 (± 6.23) years. The mean duration of DM ± SD of the patients were 3.76(± 0.78) years. The mean ± SD of the serum creatinine ± SD of the patients were 0.94(± 0.09) mg/L.The mean eGFR-Cystatin C ± SD were 109.10 (± 15.51) ml/min/m2. The meaneGFR-Cystatin C ± SD of the patients were 96.75 (± 10.91) ml/min/m2.

The cross tabulation of DTPA-GFR was done with eGFR-creatinine and eGFR-Cystatin C. The calculated sensitivity, specificity and accuracy of eGFR-creatinine were 85%, 87.2% and 85% respectively. The sensitivity, specificity and accuracy of eGFR-CystatinC were 92.3%,91.5% and 98.33% respectively. The eGFR Cystatin showed higher sensitivity, higher specificity and higher accuracy than eGFR creatinine in studied diabetic subjects. This finding is consistent with other studied[2,9]. The agreement test done between serum cystatin C and serum creatinine. The test showed K = 0.74 which indicates good agreement. Moreover, Pearson's correlation test was done. Cystatin C showed more significant correlation r = 0.78, p < 0.001 than serum creatinine, r = 0.59.p < 0.001 with DTPA-gfr in diabetic patients. This findings are consistent with other studies[10,11]. The Receiver Operator Characteristics (ROC) curves were generated by plotting the sensitivity versus specificity. Accuracy is measured by the area under the ROC curve. The area under curve (AUC) of eGFR- cystatin C is 0.88 and the AOC of eGFR-creatinine is 0.88. So the accuracy of the eGFR-Cystatin C is superior to eGFR-creatinine. Thus serum Cystatin C may be a better predictor of renal function in diabetic patients than serum creatinine[2].

In may be concluded that stimation of GFR using an appropriate method is a reliable measure of the kidney function and impairment in diabetic nephropathy. The inadequacy of the traditional markers in detecting early changes in GFR particularly monitoring the course of advanced diabetic nepfropathy calls for alternative non-invasive methods in clinical nephrology. Cystatin C seems to be an alternative and more accurate serum marker than serum creatinine for early detection of nephropathy in diabetic patients for which early treatment is important. Thus, serum Cystatin C might be a superior marker of GFR evaluation compared to creatinine and might be added to routine renal tests.

References

1. Evans TC, Cappel P. Diabetic Nephropathy. Clinical Diabetes 2000; 18:7-21.
2. Mussap M, Vestra MD, Fioretto P, Sallar A,Varagnolo M,Nosadini R, Plebani M. Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. Kidney International 2002; 61:1453-61.
3. Tan GD, Lewis AV, James TJ, Altmann P,Taylor RP, Levy JC.Clinical Usefulness of Cystatin C for the Estimation of Glomerular Filtration Rate in Type 1 Diabetes . Diabetes Care 2002; 25: 2004-9.
4. Hojs R, Bevc S, Ekart R, Gorenjak M,Puklavec L. Serum Cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. Nephrol Dial Transplantat 2006; 21:1855-62.
5. Uzun H, Ozmen KM, Taman R, Aydin S, Kalender B, Uslu E, et al. Serum Cystatin C level as a potentially good marker for impaired kidney function. Clin Biochem 2005; 38:792-8.
6. Finney H, Newman DJ, Gruber W, Merle P, Price CP. Initial Evaluation of Cystatin C measurement by particle Enhanced immunonephelometry on the behring nephelometer system. Clini Chemi 1997; 43:1016-22.
7. Murray RL, Kaplan A. Creatinine.Clin Chem 1984; 1:1261-66.
8. Premaratne E, Maclsaac RJ, Finch S,Panagiotopoulos S,Ekinci E, Jerums G. Serial Measurement of Cystatin C Are More Accurate Than Creatinine-Based Methods in Detecting Decling Renal Function in Type 1 Diabetes. Diabetes Care 2008; 31: 971-3.
9. Buysschaert M,Joudi I,Wallemacq P,Hermans MP.Comparative performance of serum Cystatin C versus serumcreatinine in diabetic subject. Diabetes and Metabolism 2003; 29: 377-83.
10. El-Agroudy AE, Sabry AA, Ghanem HA, EL-Baz A, Fakhry A, Gad HM et al. Serum Cystatin C: A good marker for evaluation of glomerular filtration rate in hepatorenal syndrome. EJ General Medicine 2004; 1: 29-35.
11. Xia LH, Bing XG, An XT. Serum Cystatin C assay for the detection of early renal impairment in diabetic patients. JC Lab Anal 2004; 18: 31-35.