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ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

Comparison of estimated glomerular filtration rate values calculated using serum cystatin C and serum creatinine

Serum sistatin C ve serum kreatinin kullanılarak hesaplanan tahmini glomerüler filtrasyon hızı değerlerinin karşılaştırılması

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ABSTRACT

Objective: In this study, we aimed to compare estimated glomerular filtration rate (eGFR) formulas based on cystatin C and serum creatinine and investigate whether the formulas can detect the renal damage at microalbuminuric level in diabetic patients.

Methods: Totally, 99 type 2 diabetic patients were included and divided into 3 groups according to 24 hour urine albumin levels as normoalbuminuric group (group 1), microalbuminuric group (group 2) and macroalbuminuric group (group 3). Creatinine clearance, Cockcroft-Gault (C-G), Modification of Diet in Renal Disease (MDRD), The Chronic Kidney Disease Epidemiology (CKD-EPI), eGFR1, eGFR2 and eGFR3 levels were calculated using formulas.

Results: There were significant differences between group 1-3 and group 2-3, but there was no significant difference between group 1 and 2 in calculated GFR levels. Cystatin C-based formulas were found to have a better correlation with creatinine clearance.

Conclusion: As a result, cystatin C-based formulas were found to predict creatinine clearance better than the other calculated GFR formulas in diabetic patients. However none of the formulas can discriminate the renal damage at microalbuminuric level. *J Clin Exp Invest 2015; 6 (2): 91-95*

Key words: eGFR, cystatin C, diabetic nephropathy, creatinine, CKD-EPI

ÖZET

Amaç: Bu çalışmada sistatin C ve/veya serum kreatinin kullanılarak hesaplanan tahmini glomerüler filtrasyon hızı (eGFR) formüllerini karşılaştırma ve bunların diyabetli hastalarda mikroalbuminürik düzeydeki hasarı tespit edip edemeyeceğinin araştırılması amaçlanmıştır.

Yöntemler: Çalışmaya tip 2 DM tanısı olan toplam 99 hasta dahil edilmiştir. Hastalar 24 saatlik idrar albumin miktarına göre normoalbuminürik (grup 1), mikroalbuminürik (grup 2), makroalbuminürik (grup 3) olarak üç gruba ayrılmıştır. Kreatinin klirensi ile Cockcroft-Gault (C-G), MDRD (Modification of Diet in Renal Disease) ve CKD-EPI (The Chronic Kidney Disease Epidemiology), eGFR1, eGFR2, eGFR3 düzeyleri formüllerle hesaplanmıştır.

Bulgular: GFR değerlerinde Grup 1-3 ve Grup 2-3 arasında anlamlı farklılık gözlenirken, Grup 1 ile Grup 2 arasında anlamlı fark gözlenmedi. Sistatin C içeren eGFR formüllerinin diğer formüllere göre kreatinin klirensi ile daha anlamlı bir korelasyon gösterdiği bulundu.

Sonuç: Diabetik hastaların takibinde Sistatin C bazlı formüllerin kullanımının, hesaplamada sistatin C kullanmayan diğer formüllere göre kreatinin klirens tahmininde daha iyi olduğu bulunmuştur. Ancak hiçbir formül böbrekteki mikroalbuminürik düzeydeki hasarı tespit edememiştir.

Anahtar kelimeler: eGFR, sistatin C, diabetik nefropati, kreatinin, CKD-EPI

INTRODUCTION

Diabetic nephropathy (DN) is one of the major chronic complications of diabetes mellitus (DM) and an important cause of morbidity and mortality in diabetic patients. In patients with diabetic nephropathy it is important to measure glomerular filtration rate (GFR) by simple, safe and fast method for early diagnosis of renal failure. GFR is accepted as the best index, which shows renal functions. Accurate

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measurement of GFR is important in the diagnosis and treatment of diabetic nephropathy. Creatinine clearance is widely used to demonstrate GFR [1-3]. However, estimated GFR (eGFR) formulas have been developed because of difficulties in collecting urine samples for 24 hours and time-consuming, expensive and exacting gold standard procedures for measurement of GFR (inulin, iohexol, 51Cr-EDTA, 99mTc-DTPA and 125I-iothalamate clearance) [4,5]. eGFR is used more than 80% of clinical laboratories in the United States [6]. The most common formulas used in the calculation of eGFR include MDRD (Modification of Diet in Renal Disease) and recently CKD-EPI (The Chronic Kidney Disease Epidemiology) equations [7,8]. It has been suggested that Cystatin C (Cys C) which is used in eGFR estimations gives more reliable results when used alone or with serum creatinine and other demographic variables [9,10]. Calculation of GFR by serum creatinine alone is affected by low or high muscle mass, diet and medications which has influence on tubular secretion. In the same way, the calculation of GFR by cystatin C alone is affected by the use of high-dose steroid [11]. In vast majority of the studies with cystatin C, it was reported that cystatin C is a better marker for demonstrating GFR and its efficiency increases especially when used with creatinine [10]. Furthermore, Cystatin C responds more quickly to the decrease in GFR; cystatin C starts to increase when GFR falls below of 80 mL/min, but creatinine starts to increase when GFR falls below of 40 mL/min [12]. For these reasons, cystatin C is suggested to be more useful for demonstrating mild to moderate renal function disorders [13].

In this study, we aimed to compare eGFR values which are calculated using cystatin C and cystatin C-serum creatinine combination in type 2 diabetic patients grouped according to their 24-hour urinary albumin levels and investigate whether the formulas calculated during routine biochemistry measurements can detect the renal damage in microalbuminuric level.

METHODS

Patients

99 patients with type 2 DM diagnosed according to WHO (World Health Organization) criteria, admitted to Ankara Numune Education and Research Hospital as outpatients were included in this study. The patients without renal dysfunction (normal serum urea and creatinine levels, without uremic complications, osteodystrophy and edema, etc.) were enrolled in the study. Before the study the patients were informed. The demographic data of patients such as age, gender, height, weight, age of disease and the treatment that patients receive for diabetes and accompanying diseases (macrovascular diseases, retinopathy, neuropathy, hypertension, hyperlipidemia) were recorded. 24-hour urine was collected for the detection of albuminuria and calculation of creatinine clearance. The patients were informed about the collection of 24-hour urine.

Patients were divided into 3 groups according to microalbumin levels. Those who have 24-hour urinary albumin <30 mg/day, 30-300 mg/day and >300 mg/day composed the normoalbuminuric group (Group 1); microalbuminuric group (Group 2), and macroalbuminuric group (Group 3); respectively.

This study was approved by the Local Ethical Committee of Ankara Numune Education and Research Hospital.

Blood and urine samples and measurements

24 hour-urine samples were collected from the patients without renal dysfunction for the calculation of albuminuria and creatinine clearance. Fasting blood samples were taken from the patients after clinical examinations. Sera were obtained by centrifuging of blood samples for 10 minutes at 1500 g. Creatinine levels were determined with Beckman Coulter DXC 800 (Fullerton, CA, USA) autoanalyzer via Jaffe Method. For cystatin C, the sera obtained from blood samples were stored at-20°C. Volume of 24 hour-urine samples were recorded. Albumin and creatinine levels were analyzed at the same time of the 24-hour urine collection in Beckman Coulter DXC800 (Fullerton, CA, USA) autoanalyzer. Microalbumin and creatinine levels were determined at the same day with Beckman Coulter DXC 800 auto analyzer with original reagents using immunoturbimetric method. Cystatin C was measured with a nephelometric DakoCytomation® reagent (Immage, Beckmann Coulter, USA).

The eGFR values of patients were calculated according to formulas of Cockcroft-Gault (C- G), MDRD and CKD-EPI, eGFR 1, eGFR 2, eGFR 3 for this study (Table 1).

Statistical Analysis

Results of the study were evaluated statistically by “The Statistical Package for Social Science for Windows (SPSS v18)” program. The conformity of continuous variables to normal distribution was test-

ed with Kolmogorov-Smirnov test. The descriptive statistics of continuous variables were expressed as mean ± SD or median (min-max). The presence of a statistically significant difference between the groups in terms of continuous variables was examined with ANOVA for parametric and Kruskall-Wallis test for non-parametric variables. For the significant ($P < 0.05$) analytes, the Student t test for parametric and Mann-Whitney test for nonparametric variables was performed. Pearson correlation analysis was used for correlation of the variables. The correlation between the calculated formulas was assessed by Spearman's Rho correlation analysis. A p value less than 0.05 was considered statistically significant.

Table 1. GFR calculation formulas

Formula of Creatinine Clearance (mL/min/1.73 m^2)	[Urine creatinine (mg/dL) × Daily urine volume (mL)] / [Plasma creatinine (mg/dL) × 1440]
MDRD (Modification of Diet in Renal Disease) formula (mL/min/1.73 m^2) [16, 21]	186.3 ×(Serum Creatinine)$^{-1.15}$ ×(Age)$^{-0.203}$ × 0.742 (in women)
Cockcroft-Gault (C-G) Formula (mL/min/1.73 m^2) [16, 21]	[(140-age) × lean body mass (kg)] / [72× Serum Creatinine] (The result is multiplied by a factor of 0.85 for women)
The Chronic Kidney Disease Epidemiology Collaboration Formula (CKD-EPI) (mL/min/1.73 m^2) [8]	[141×min(Serum Creatinine/κ,1)$^{\alpha}$ × max (Serum Creatinine/κ,1) $^{-1.209}$×0.993^{age}]× 1.018 [for women]× 1.159[for African American] (κ: 0.7 for women, 0.9 for men, α: -0.329 for women , -0.411 for men)
eGFR* 1 Formula (mL/min/1.73 m^2) [16, 21]	76.7×(- 0.105+1.13×Serum Cys C**) $^{-1.19}$
eGFR* 2 Formula (mL/min/1.73 m^2) [16, 21]	127.7×(- 0.105+1.13×Serum Cys C**)$^{-1.17}$
eGFR* 3 Formula (mL/min/1.73 m^2) [16, 21]	177.6 × (Serum creatinine)$^{-0.65}$ × (-0.105 +1.13 × Serum cystatin C)$^{-0.57}$×Age$^{-0.20}$ (×0.82, women)

*:estimated glomerular filtration rate, **:cystatin C

RESULTS

Patients with type 2 diabetes mellitus were 58 female and 41 male. The mean age was 55.82 ± 9.87. The GFR values which were calculated with CKD-EPI, eGFR 1, eGFR 2, eGFR 3, Creatinine clearance, MDRD and C-G GFR formulas were found to be significant between groups (p<0.001). There was no significant difference between Group 1 and Group 2 in terms of CKD-EPI, eGFR 1, eGFR 2, eGFR 3, Creatinine clearance, MDRD and C-G GFR levels (p>0.05). CKD-EPI, eGFR 1, eGFR 2, eGFR 3, Creatinine clearance, MDRD, C-G GFR levels were significantly higher in Group 1 than Group 3 (p<0.001). CKD-EPI, eGFR 1, eGFR 2, eGFR 3, Creatinine clearance, MDRD, C-G GFR levels were significantly higher in group 2 than group 3 (p<0.001). The data of GFR values are shown in Table 2.

Table 2. Comparison of the levels of creatinine clearance and eGFR calculated by formulas in type 2 DM patients

	Group 1 (n = 34)	Group 2 (n = 43)	Group 3 (n = 22)	p value
Age (years)	54.58±10.0	55.76±10.17	57.86±7.91	0.469
CKD-EPI[1] (mL/min)	98.17 (56.82-121.45)*	98.00 (60.47-132.55)*	66.57 (26.37-80.87)*	<0.001[a,b]
eGFR[2] 1 (mL/min)	84.99±14.20	79.37±16.48	53.98±15.39	<0.001[a,b]
eGFR[2] 2(mL/min)	79.30±14.87	74.91±16.17	50.63±14,69	<0.001[a,b]
eGFR[2] 3 (mL/min)	89.84±16.62	88.39±19.05	56.82±12.47	<0.001[a,b]
Creatinine clearance (mL/min)	109.93 (80.30-128.55)*	116.87 (74.60-277.59)*	81.74 (23.20-174.60)*	<0.001[a,b]
MDRD[3] (mL/min)	97.04±21.13	100.01±27.14	63.96±12.63	<0.001[a,b]
C-G[4]GFR (mL/min)	110.69±29.40	116.43±43.08	75.75±18.70	<0.001[a,b]

*Mean ± SD, median (minimum-maximum)

[a] : The difference between group 1 and group 3 is statistically significant.

[b] : The difference between group 2 and group 3 is statistically significant.

1: The Chronic Kidney Disease Epidemiology, 2: Estimated Glomerular Filtration Rate, 3: Modification of Diet in Renal Disease, 4: Cockcroft-Gault

In the correlation analysis, a significant correlation was observed between creatinine clearance and eGFR 1 (r=0.353, p=0.041) and eGFR 2 (r=0.426, p=0.012) in group 1. In group 2, eGFR 1 (r=0.399, p=0.008), eGFR 2 (r=0.394, p=0.009), eGFR 3 (r=0.380, p=0.012), C-G GFR (r=0.390, p=0.010) and CKD-EPI (r=0.316, p=0.039) formulas were determined to be associated with creatinine clearance. eGFR 1 (r=0.846, p<0.001), eGFR 2 (r=0.839, p<0.001), eGFR 3 (r=0.762, p<0.001) and C-G GFR (r=0.577, p=0.005) formulas were found to be associated with creatinine clearance in group 3. The correlation results of GFR values are shown in Table 3.

In normoalbuminuric group (group 1), there was a significant difference in creatinine clearance and the estimated GFR values of MDRD, CKD-EPI, eGFR 3, eGFR 2 and eGFR 1 formulas (p<0.05). However there wasn't a difference between creatinine clearance and estimated GFR of C-G formula (p=0.831) (Table 4).

Table 3. The correlation coefficients and significance degrees of creatinine clearance and eGFR calculated by formulas in Type 2 DM patients

(mL/min)	Group 1 (n = 34)		Group 2 (n = 43)		Group 3 (n = 22)	
	r	p	r	p	r	p
CKD-EPI	0.147	0.407	0.316	0.039	0.351	0.110
eGFR1	0.353	0.041	0.399	0.008	0.846	<0.001
eGFR2	0.426	0.012	0.394	0.009	0.839	<0.001
eGFR3	0.248	0.157	0.380	0.012	0.762	<0.001
MDRD	0.078	0.663	0.244	0.115	0.222	0.321
C-G GFR	0.142	0.424	0.390	0.010	0.577	0.005

CKD-EPI: The Chronic Kidney Disease Epidemiology, eGFR: Estimated Glomerular Filtration Rate, MDRD: Modification of Diet in Renal Disease, C-G: Cockcroft-Gault

Table 4. Comparison of the difference of the estimated GFR levels and creatinine clearance levels within groups

	Group 1 p value	Group 2 p value	Group 3 p value
MDRD	0.026	<0.001	0.005
C-G	0.831	0.322	0.140
CKD-EPI	0.001	<0.001	0.006
eGFR 3	<0.001	<0.001	<0.001
eGFR 2	<0.001	<0.001	<0.001
eGFR 1	<0.001	<0.001	<0.001

MDRD: Modification of Diet in Renal Disease, C-G: Cockcroft-Gault, CKD-EPI: The Chronic Kidney Disease Epidemiology, eGFR: Estimated Glomerular Filtration Rate

DISCUSSION

GFR is accepted to be the best index to reflect renal function. In GFR estimation, endogenous creatinine clearance provides more accurate results compared with serum creatinine [14,15]. Other gold standard measurement methods of GFR such as exogenous inulin, iohexol, iothalamate or the use of radioactive markers are invasive and expensive methods that can cause waste of time and also have potential complications [15].

Cystatin C is an endogenous 13-kDa protein which is produced in a constant rate regardless of muscle mass and cleared by filtration in the glomeruli, its small amount is reabsorbed and catabolized by tubular epithelial cells, then excreted into urine. It has a weaker relationship with age, gender and race than creatinine [16].

In the study of Bevc et al, eGFR values in obese patients with type 2 DM were calculated by Cockcroft-Gault, MDRD, creatinine-based CKD-EPI, creatinine and cystatin C-based CKD-EPI [17]. The results were compared with Cr-EDTA which is accepted as the gold standard. Although all formulas have shown to have significant correlations with Cr-EDTA clearance, it was shown that eGFR obtained by Cockcroft-Gault formula in ROC analysis had minimum area under the curve. The reason for this was thought to be related with excess weight. Also cystatin C measurements were performed and it was shown that diagnostic performance of cystatin C is as good as creatinine-based formulas [17].

Xin Du et al. compared CKD-EPI, cystatin C-based GFR, combination of cystatin C and creatinine-based (Cys C-Scr) GFR formulas in 111 patients with chronic renal failure by standard renal dynamic analysis method with Tc-DTPA [18]. A positive correlation was found between standardized GFR and CKD-EPI (r=0.467, P<0.001), Cys C GFR (r = 0.747, P<0.001), Cys C-Scr GFR (r=0.785, P<0.001) formulas [18]. In our study, a significant relation was not found between CKD-EPI values of each 3 groups and creatinine clearance. It was observed that the Cys C-based eGFR 1 formula had a higher correlation coefficient with creatinine clearance than the other eGFR formulas. Also Feng et al. reported that the formula using Cystatin C alone is more effective than the formula including Cystatin C, creatinine and age. Therefore Cystatin C can be useful for evaluating renal function instead of creatinine [5]. Marwyne et al. studied Cys C based and creatinine based eGFR equations in overweight and obese subjects and compared eGFR values with

99mTc-DTPA GFR. They found that Cys C based equation is more correlative than creatinine based equation. Also they reported that Cys C based equation has better accuracy, sensitivity and specificity in abnormal GFR levels (<90 mL/min/1.73 m^2 or >120 mL/min/1.73 m^2) [19].

Camargo et al. compared GFR values measured by Cr-EDTA with eGFR values calculated by MDRD and CKD-EPI formulas in Type 2 DM patients. It was determined that eGFR values calculated by both formulas were lower than GFR measured by Cr-EDTA in both healthy and patient groups. They concluded that even if CKD-EPI formula seems better than MDRD formula in healthy group, additional corrections are needed for the use of these formulas in diabetic patients [20,21]. In our study, while a significant difference between group 1 and 3, between group 2 and 3 were observed in terms of CKD-EPI and MDRD formulas, no significant difference was determined between group 1 and 3. It was found that CKD-EPI values of all 3 groups were correlated better with creatinine clearance compared with MDRD formula.

In our study it was found that correlations between eGFR calculation methods and creatinine clearance were better when compared with formulas which do not contain cystatin C (Table 3).

In conclusion, Cystatin C-based formulas were found to predict creatinine clearance better than the other calculated GFR formulas in diabetic patients however none of the formulas can discriminate the renal damage in microalbuminuric level.

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