



Determination of Cystatin C as a marker of renal function in normoalbuminuric patients with type 2 Diabetes Mellitus. A multicenter study.

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Abstract

Introduction: Early identification of impaired renal function is crucial in diabetic patients. Clinically, serum cystatin C could be the most sensitive indicator of glomerular filtration rate. The objective of the present study was to propose the use of cystatin C as a routine test to evaluate renal function in type 2 diabetic patients.

Methods: An observational cross-sectional study on 115 normoalbuminuric type two diabetic patients, establishing the glomerular filtration rate with cystatin C values and the Cockcroft-Gault formula. Those with a glomerular filtration rate < 60 mL/min underwent 24-hour urine creatinine clearance. We compared the results with the statistical program SPSS 17, presenting the data in contingency tables with risk analysis and significance test of X^2 .

Results: The glomerular filtration rate obtained with cystatin C had an average value of 99 ± 19.07 ml/min/1.73 square meters of body surface (smbs). The Cockcroft-Gault value was 79.85 ± 23.63 ml/min/1.73 smbs, reflecting a better correlation with creatinine clearance in 24-hour urine, which was 74.95 ± 37.41 ml/min/1.73 smbs. There was no significant relationship between both parameters when relating the values to the 24-hour urinary creatinine clearance ($P = 0.14$).

Conclusions: The glomerular filtration rate with cystatin C presents a significant dispersion in the correlation with the creatinine clearance in the urine for 24 hours; this fact, together with various factors that influence its variability, and the higher cost of its determination, make its use to assess renal function little justifiable.

Keywords:

MESH: Cystatin C; Renal Insufficiency, Chronic; Diabetes Mellitus, Type 2; Glomerular Filtration Rate; Albuminuria.

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The evaluation of kidney function in diabetic patients is key to the early identification and proper management of diabetic nephropathy, but during the initial stages, its progression is silent, and its detection with routine tests is difficult. Knowing that, even when kidney damage cannot be reversed, its deterioration can be reduced with early intervention in blood pressure control, protein restriction in the diet, and stricter control of glycemia, avoiding complications [1]. The best method to assess renal function is the glomerular filtration rate, which is reduced before the onset of symptoms. Although its estimation is accurate through the administration of exogenous substances, these are difficult to use in daily clinical practice. Currently, the use of the Cockcroft-Gault and MDRD formulas is advocated to estimate the glomerular filtration rate. However, these formulas are subject to variables, including weight, muscle mass, age, sex, and race; on the other hand, the quantification of endogenous substances such as protein in urine and creatinine and its clearance in 24-hour urine is the most widely used to assess the glomerular filtration rate (GFR). Despite its great utility, the latter has limitations due to the sample collection technique [2]. Most medical societies recommend using microalbuminuria levels to monitor renal function in diabetic patients; however, normoalbuminuric individuals are not exempt from developing it, and others with microalbuminuria are not necessarily destined to present nephropathy [3]. Serum cystatin C has recently been proposed as a marker of renal function, and this is a nonglycosylated protein produced by all nucleated cells in the body, freely filtered by the glomeruli, and reabsorbed and metabolized in the renal tubules. Its serum levels are not influenced by sex, age, race, or body mass index, contrary to what happens with serum creatinine, making the latter a parameter insensitive to slight changes in GFR due to its wide reference range [4].

Materials and methods

Study design

This multicenter study is observational and cross-sectional.

Scenery

The study was carried out at the Rodríguez Zambrano Hospital Diabetic Association in Manta, the Verdi Cevallos Balda Hospital in Portoviejo and the IESS Teodoro Maldonado Carbo Regional Hospital in the city of Guayaquil. The study period was from February 01, 2010, to April 30, 2010. The study ended on July 30, 2010.

Participants

Voluntary patients with confirmed type 2 diabetes mellitus diagnosis, with an evolution of at least two years, with microalbuminuria less than 20 µg/min, and aged between 18 and 95 years were included. Patients with renal insufficiency of any etiology, thyroid disease, known cardiovascular disease (heart failure, valvular disease), those with a history of renal transplantation, neoplasms, or those treated with glucocorticoids or statins were excluded from the study.

Variables

The variables studied were age, weight, sex, pathological history, pharmacological habits, and time of evolution of the disease.

Data sources/measurements

For the study, blood and urine samples were taken, fasting glycemia levels were determined with the enzymatic method of glucose oxidase, serum creatinine was determined with the Jaffé colorimetric method, and both tests were performed with a Hitachi 917 analyzer. Microalbuminuria was quantified in a urine sample at random using the immuno-chemiluminescence method processed on the Inmulite I analyzer. Those patients who had microalbuminuria levels within normal ranges underwent cystatin C quantification using the particle-enhanced immunoturbidimetry method on the Hitachi 917 analyzer. Then, the glomerular filtration rate was calculated according to cystatin C values and with the Cockcroft-Gault formula, and those patients who had a glomerular filtration rate <60 ml/min had creatinine clearance in 24-hour urine.

Biases

The results were validated by the technical operators and by a superior validation confirmation area (clinical laboratory doctor).

Studio size

The sample was probabilistic, with a confidence interval of 95% and an estimated population size of 8%, with an error of 5%; the sample calculation was 115 cases.

Quantitative variables

With the creatinine clearance obtained with the Cockcroft-Gault formula, the measurement with cystatin C, and the measurement with 24-hour urinary creatinine clearance, comparative calculations were made using the averages and standard deviation.

Statistical analysis

The statistical program used was SPSS 17 (Chicago: SPSS Inc.). Linear regression was performed between the glomerular filtration rate obtained using the formula for cystatin C, Cockcroft-Gault, and creatinine clearance in 24-hour urine.

Results

Participants

A total of 115 patients entered the study. The diagram of the participants is presented in Figure 1.

Characteristics of the study population

The results obtained in the study show a more significant number of female patients (82.6%) compared to the male population, which represented 17.4% of the patients, with a significance of $P = 0.035$, as in this population, 57.4% are under 60 years old. The average age of the patients was 60.83 ± 10.13 . (Table 1-2).

A total of 57.4% of the patients presented elevated glucose levels. A total of 34.8% of the patients presented values above



the cutoff point in creatinine, while 69.6% did so for cystatin C. The mean value of creatinine was 0.79 ± 0.15 mg/dL, and that of cystatin C was 0.88 ± 0.19 mg/d/dL; however, the cutoff point for creatinine was higher than that for cystatin C (0.84 vs. 0.76 in women and 0.86 vs. 0.85 in men). (Table 1). The glomerular filtration rate obtained with cystatin C had an average value of 99 ± 19.07 , and the Cockcroft-Gault ml/min was 79.85 ± 23.63 ; the latter showed a better correlation with the creatinine clearance in 24-hour urine obtained by a value of 74.95 ± 37.41 ml/min (Table 1). When comparing the mean values of creatinine and cystatin C in terms of age and sex, higher levels of both were observed in patients between 70-79 years of age, with cystatin C surpassing creatinine in this group (1.06 ± 0.20). vs 0.90 ± 0.15 , $P < 0.001$). Higher values of creatinine than cystatin C were observed in the male population,

no significant difference between the two (0.86 ± 0.12 vs. 0.95 ± 0.10), while in women, the values of cystatin C exceeded the values of creatinine (0.86 ± 0.12 vs. 0.95 ± 0.10) (Figure 2).

However, although cystatin C values were higher than creatinine values in most cases, they remained within the values considered normal. ROC curves were constructed to determine the diagnostic capacity of the estimated glomerular filtration rate using the cystatin C and Cockcroft-Gault formulas, where it was shown that the Cockcroft-Gault formula had a greater area under the curve than the first, with greater specificity without presenting significant differences (Figure 3). When relating the values of the glomerular filtration rate obtained with cystatin C and the creatinine clearance in 24-hour urine, no significant relationship was found between both parameters ($P = 0.14$) (Figure 4).

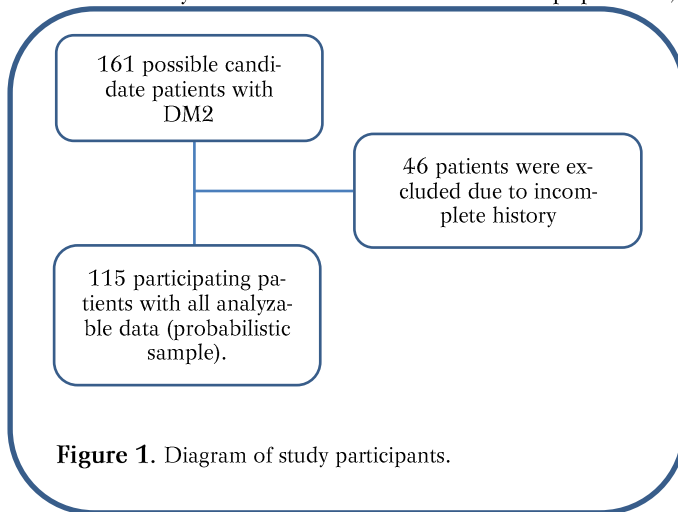


Figure 1. Diagram of study participants.

Table 1. Primary variables of the study.

Variable	Mean \pm SD
Age (Years)	60.18 ± 10.1
Glucose (mg/dL)	143.6 ± 70.9
Creatinine (mg/dL)	0.79 ± 0.15
Microalbuminuria (mg/dL)	5.92 ± 4.2
Cystatin C (mg/dL)	0.88 ± 0.19
Creatinine clearance (ml/min/1.732)	74.95 ± 37.41
GFR- Cystatin C (ml/min/1.732)	99.63 ± 19.1
Cockcroft-Gault (ml/min)	79.85 ± 23.63
Weight (kg)	64.17 ± 12.3
Size (m)	1.53 ± 0.083
BMI (Kg/m ²)	27.23 ± 4.43

SD: Standard deviation. TFG. Glomerular filtration rate. BMI: Body mass index.

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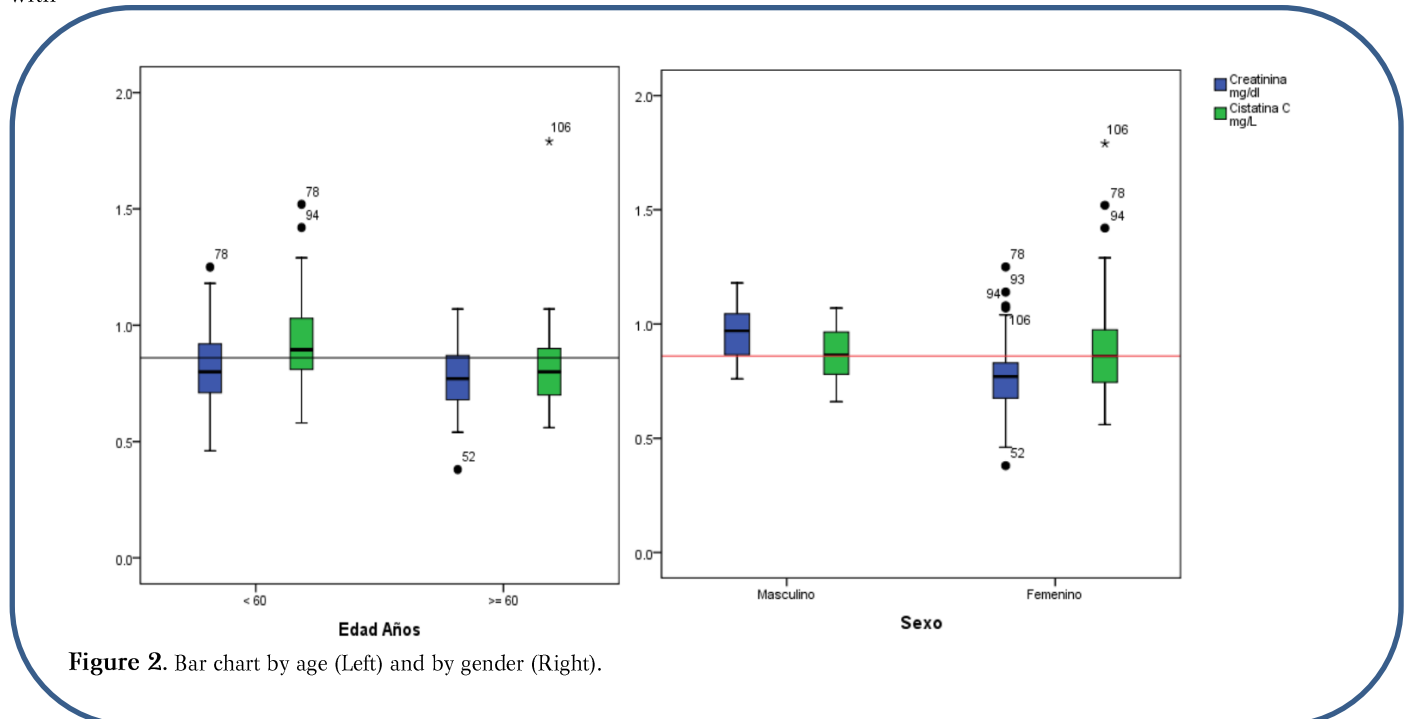


Figure 2. Bar chart by age (Left) and by gender (Right).

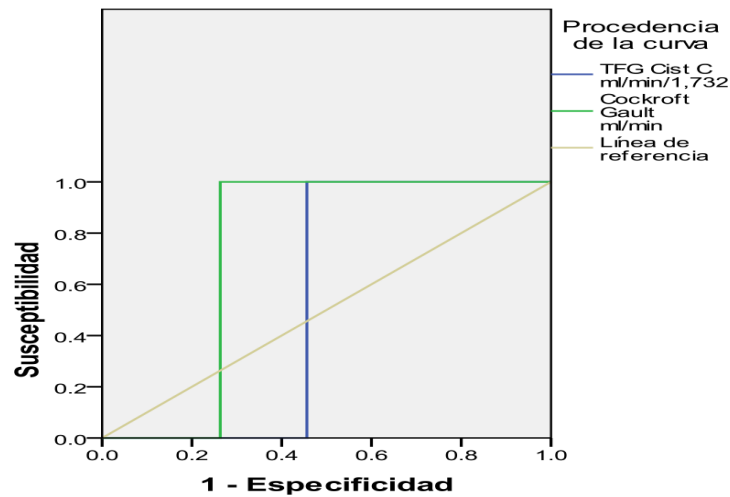


Figure 3. COR chart. Cystatin: Area 0.544 P=0.88. Cockcroft-Gault, Area 0.737, P=0.41

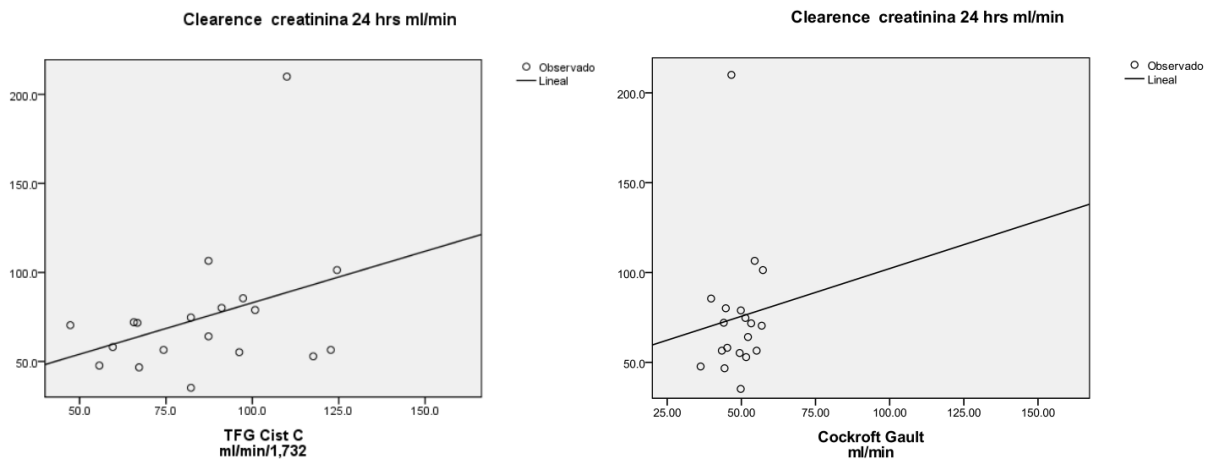


Figure 4. Scattergrams of creatinine clearance with Cystatin C (Left) and Cockcroft –Gault (right) R=0.82 P=0.738.

Age	N=115	Cystatin C	Creatinine	P
30 to 39	3	0.67 ± 0.06	0.75 ± 0.34	NS
40 to 49	9	0.74 ± 0.12	0.72 ± 0.10	NS
50 to 59	37	0.85 ± 0.19	0.78 ± 0.13	0.041
60 to 69	44	0.87 ± 0.14	0.78 ± 0.14	0.012
70 to 79	20	1.06 ± 0.20	0.90 ± 0.15	<0.01
≥ 80 years	2	0.95 ± 0.03	0.74 ± 0.11	0.28
Sex				
Men	20	0.86 ± 0.12	0.95 ± 0.10	0.38
Woman	95	0.88 ± 0.20	0.76 ± 0.14	<0.01
Total	115	0.88 ± 0.19	0.79 ± 0.15	<0.01

Creat: Serum creatinine. HD: Hemodialysis.

Discussion

Renal deterioration in its initial stage is asymptomatic, so its detection represents a significant challenge for doctors. The glomerular filtration rate is used worldwide as the parameter for assessing renal function in the population at risk, as in the case of diabetic patients; however, it is currently estimated using serum creatinine levels, which are affected by some variables and are insensitive to slight decreases in the glomerular filtration rate, being altered when the glomerular filtration rate drops to 50% of its average value [5- 7]. This limitation makes it essential to look for more sensitive markers such as cystatin C, which, according to evidence from recent studies, exceeds serum creatinine in this area. This study showed a small variability between sexes for the determination of cystatin C that was



insignificant, so we conclude that the test is not dependent on sex.

Regarding age, higher levels of cystatin C were observed after 40 years of age, which coincides with what has been described in the literature, showing the highest values in subjects over 70 years of age due to the physiological decrease in renal function. Despite this, cystatin C is not a test whose value is dependent on age. Regarding serum creatinine levels, they showed similarity at all ages, increasing slightly in patients between 70 and 80 years of age and a slight decrease in patients over 80 years of age, with a higher mean in males. Serum creatinine varies according to age, sex, and muscle mass and is not a sensitive test to determine small decreases in the glomerular filtration rate in asymptomatic patients [8, 9]. Studies suggest that the serum concentration of cystatin C correlates well with creatinine clearance in 24-hour urine. This method is subject to inaccuracies in the collection technique and various analytical interferences, which has prompted various investigations in recent years assessing this new renal marker; however, in this study, these values did not present a significant correlation [10, 11].

Regarding work with diabetic patients, most type 2 diabetic patients, the studies are controversial. Among others, Mussap et al. [12] commented on the greater specificity of cystatin C in the evaluation of renal function, highlighting the lack of precision of traditional markers in detecting early changes in glomerular filtration rate and in monitoring the course of diabetic nephropathy. In this investigation, specificity values for cystatin C of 61% were presented, while creatinine presented a specificity of 85%, resulting in more convenience, which can be seen in the ROC curve; however, neither of them presented good sensitivity. (Figure 3). The results of the present study show that, although free from influences by age and sex, the glomerular filtration rate with cystatin C presents a large dispersion in the correlation with the 24-hour urinary creatinine clearance. This fact, together with the uncertainty of the factors that influence its variability and the higher cost of its determination, makes its use as an estimator of glomerular filtration hardly justifiable, in contrast with what was stated by Murray, who indicated that the monitoring of the values of cystatin C in at-risk patients could anticipate creatinine by one or two days in the diagnosis of acute renal failure and slow down its progression [13, 14].

The limitation of our study is the use of ACE inhibitors in our patients since there are works that mention that these drugs produce alterations in cystatin C levels in patients. Unfortunately, our study population was diabetic patients, of whom some of them were taking a drug from this group, as the diabetes clinical guidelines refer to it; in addition, many of them were hypertensive, and in our environment, the use of Enalapril, due to its low cost, is among the drugs of choice for this pathology, not so much in other countries where studies were carried out with cystatin C in people with diabetes receiving another class of antihypertensives; therefore, we conclude that studies should be carried out with a larger sample and take into consideration the use of ACE inhibitors and their influence on the assessment of glomerular filtration rate with cystatin C. Another significant limitation of our study was the time it was carried out since the ideal would be to follow up our patients with serial determinations of serum cystatin C and thus

adequately assess their behavior when renal function begins to decline.

Conclusions

The glomerular filtration rate with cystatin C shows a large dispersion in the correlation with the creatinine clearance in 24-hour urine; this fact, together with various factors that influence its variability and the higher cost of its determination, make its use to assess renal function little justifiable.

Abbreviations

SD: Standard deviation.
ACE inhibitor: angiotensin-converting enzyme inhibitor.
BMI: Body mass index.
ROC: receiver operating characteristics.
TFG: Glomerular filtration rate.

Supplementary information

Supplementary materials have not been declared.

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Author contributions

Juan Manuel Alcívar Vásquez: Conceptualization, Data Curation, Formal Analysis, Fundraising, Research, Methodology, Project Management, Resources, Software, Writing – original draft.

Carlos Alberto Puig Gilbert: Conceptualization, Data Curation, Formal Analysis, Fundraising, Research, Methodology, Project Management, Resources, Software, Writing – original draft.

Jeany Wong Lama: Conceptualization, Data Curation, Formal Analysis, Fundraising, Research, Methodology, Project Management, Resources, Software, Writing – original draft.

Miguel Angel Flor Rodríguez: conceptualization, supervision, validation, visualization, and writing: review and editing.

All authors read and approved the final version of the manuscript.

Financing

The authors provided research expenses.

Availability of data or materials

The data sets generated and analyzed during the current study are not publicly available due to participant confidentiality but are available from the corresponding author upon reasonable academic request.

Statements

Ethics committee approval and consent to participate

The bioethics committee approved the study of the Faculty of Medicine of the Universidad Católica Santiago de Guayaquil.

Consent to publication

This does not apply when images or photographs of the physical examination or radiography/tomography/MRI of patients are not published.

Conflicts of interest

The authors report having no conflicts of interest.

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