RESEARCH ARTICLE

A comparison of cystatin C concentrations between patients with chronic interstitial nephritis and glomerular diseases

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Aims: Cystatin C have shown to be a renal function parameter with higher sensitivity and specificity than serum creatinine. In tubular diseases, cystatin C degradation and an increase in its urinary elimination would be observed. We have tried to define if different kinds of kidney diseases may significantly affect the serum levels of cystatin C.

Design and Methods: Four hundred and four patients were studied: 249 men and 155 women, mean age was 64.3±10.1 years. Patients were classified into three groups: (1) Chronic interstitial nephropathy (CIN), (2) Glomerular nephropathy (GN), and (3) Non-CKD patients (control). GFR was estimated through the CKD-EPI equation and the Hoek formula.

Results: Median of serum creatinine levels was higher in CIN group than in GN and control groups. Median cystatin C levels were lower in control group compared with CIN and GN groups. No significant differences were found between CIN group and GN group. Nevertheless, the cystatin/creatinine rate was lower in the CIN group patients (0.94, 0.81-1.11) than in the GN group (1.02, 0.85-1.25) as well as the control group (1.02, 0.88-1.20). The cystatin C-estimated GFR/creatinine-estimated GFR rate was higher in the CIN group (1.18, 1.03-1.36) than in the GN patients (1.03, 0.88-1.21) and control ones (1.07, 0.88-1.20).

Conclusions: Patients with CIN had lower serum levels of cystatin C defined as cystatin C/creatinine rate when they were compared with GN subject and control ones. In the same way, the index between cystatin C-estimated GFR and creatinine-estimated GFR was higher in CIN patients.

KEYWORDS

chronic interstitial nephropathy, chronic kidney disease, cystatin C

1 | INTRODUCTION

Cystatin C (CysC) is an interesting new marker for the estimation of GFR. It does offer several advantages over creatinine or other similar molecular weight proteins. CysC is produced by all nucleated cells in the human body and because the protein is coded by a housekeeping gene, (i.e. a gene expressed both constitutively and in an unregulated manner), CysC is considered to be constantly produced.¹ After being filtered

without restriction by the glomeruli because of its low-molecular mass and absence of protein binding, CysC is entirely reabsorbed by the proximal tubules, where it is almost entirely catabolized.² Current KDIGO Guidelines for chronic kidney disease (CKD) suggests measuring of CysC in adults with GFR 45–59 mL/min/1.73 m² who do not have markers of kidney damage if confirmation of CKD is required.³

Cystatin C has shown to be an early and sensitive marker of acute renal failure. Creatinine serum concentrations are an insensitive

marker of acute kidney injury, and the speed of an increase from baseline depends on the magnitude of the acute injury and pre-existing kidney functional reserve. A wide range of inter-individual concentrations means that single time-point determinations are difficult to interpret, and acute kidney injury may not manifest as a detectable increase in serum creatinine concentrations until at least 24–48 h after the primary insult. Contrariwise, serum and urinary cystatin C concentrations are closely related to kidney function and, for example, in acute tubular necrosis allow better prediction of the need for renal replacement therapy than serum creatinine concentrations.⁴

Cystatin C is a strong inhibitor of cysteine proteinases which is freely filtered by the kidney glomerulus and is reabsorbed by the tubules, where it is almost totally catabolized, with the remainder then eliminated in urine. In tubular diseases, it has been suggested that cystatin C degradation would be reduced and consequently an increase in its urinary elimination would be observed. In fact, Conti et al.⁵ have reported that urine cystatin C concentrations obtained from patients with kidney tubular disease were significantly higher than those for controls and glomerular disease patients. Therefore, the etiology of CKD might influence the serum levels of cystatin C. We have tried to define if different kinds of kidney disease may significantly affect the serum levels of cystatin C.

2 | DESIGN AND METHODS

Four hundred and four patients were studied: they were 249 men and 155 women, mean age was 64.3±10.1 years. Only Caucasian patients were included in the study. Patients were classified into three groups: (1) Chronic interstitial nephropathy (CIN), (2) Glomerular nephropathy (GN), and (3) Non-CKD patients (control). Table 1 depicts the groups characteristics. Control subjects were recruited from patients attending the outpatient office without signs of renal disease in the evaluation.

Serum CysC was measured using a BNII nephelometer (Dade Behring Inc., Deerfield, IL, USA) that used a particle-enhanced immunonephelometric assay (N-Latex Cystatin C). The assay range being 0.195–7.330 mg/L, with the reference range for young healthy individuals reported as 0.53–0.95 mg/L. The cut-off for the highest quartile of serum cystatin distribution was 1.05 mg/L and this was the limit used to define high plasma cystatin levels. Serum creatinine was measured through standardized Jaffé method (IDMS) and GFR was estimated from serum creatinine using the CKD-EPI equation (for every

sex).⁶ The Hoek formula was used to estimate the GFR from cystatin C.⁷ It was considered as normal serum creatinine <1.4 mg/dL for men and <1.2 mg/dL for women. Decreased estimated GFR was defined as a value <60 mL/min. The amount of Urinary albumin excretion over a period of 24 hours was measured from the samples and patients were categorized using KDIGO definitions.⁵

Results are expressed as mean±1 standard deviation. All statistical tests were two-sided. *P* values lower than 0.05 were considered as significant. For comparisons between groups, Student “*t*” test for paired samples was used for continuous variables and Chi-square test for categorical variables. Since Kolmogorov-Smirnov *Z* test found that CysC, microalbuminuria and estimated GFR did not follow a normal distribution, Mann-Whitney “*u*” test was used to compare values. These parameters have been expressed as median (IR, interquartile range). The statistical analysis was developed with the package SPSS 21.0 (IBM, Armonk, New York, USA).

All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in this survey and the study design was approved by our hospital Ethics Committee.

3 | RESULTS

Median of serum creatinine levels was higher in CIN and GN groups when compared with control group (*P*<.001, Mann-Whitney test) (see Table 1). The difference between both groups also was statistically significant being the median of serum creatinine higher in CIN group (*P*=.004, Mann-Whitney test). Median cystatin C levels were lower in control group (*P*<.001 vs CIN and GN groups, Mann-Whitney test). No significant differences were found between CIN group and GN group. All values are summarized in Table 2.

Nevertheless, the cystatin/creatinine rate was lower in the CIN group patients (0.94, RI 0.81–1.11) than in the GN group (1.02, RI 0.85–1.25, *P*=.038 Mann-Whitney test) as well as the control group (1.02, RI 0.88–1.20, *P*=.017, Mann-Whitney test).

The median of CKD-EPI-estimated GFR and Hoek-estimated GFR was lower in the CIN and GN groups than in the control group (*P*<.001, Mann-Whitney test). In the same way, creatinine-estimated GFR was lower in the CIN group than in the GN group (*P*<.001). All values are

	CIN	GN	Control
N	128	122	154
Sex (men/women)	76/52	83/39	90/64
Age	66.0±11.4	63.4±9.8	63.7±9.0
Creatinine (mg/dL)	1.81 (1.37–2.45) ¹	1.52 (0.92–2.17)	0.83 (0.68–1.00)
Albuminuria (mg/d)	68.1 (12.5–238) ¹	559 (218–1198)	9.20 (4.20–20.2)

TABLE 1 Comparative sample characteristics of CIN, GN, and Control groups

See abbreviations and significations in the text.

¹*p* < 0.01 Mann-Whitney test.

TABLE 2 Differences in cystatin and GFR in CIN, GN, and controls

	CIN	GN	Control
Cystatin C	1.79 (1.23-2.27) ¹	1.54 (1.05-2.16)	0.79 (0.69-1.01)
Cystatin C/Creatinine	0.94 (0.81-1.11) ²	1.02 (0.85-1.25)	1.01 (0.85-1.18)
CKD-EPI GFR	37.7 (24.3-50.8) ¹	47.5 (31.1-74.9)	92.3 (71.0-110)
Cystatin C GFR	39.6 (30.8-58.8) ¹	48.5 (33.0-72.9)	96.8 (75.2-112)
Cystatin C GFR/Creatinine GFR	1.18 (1.03-1.36) ¹	1.04 (0.89-1.22)	1.06 (0.88-1.18)

See abbreviations and significations in the text.

¹ $p < 0.01$ Mann-Whitney test, ² $p < 0.05$ Mann-Whitney test.

summarized in Table 2. The cystatin-estimated GFR was also higher in the GN group than in the CIN group ($P=.01$, Mann-Whitney test).

Contrariwise, the estimated GFR/creatinine-estimated GFR rate was higher in the CIN group (1.18, RI 1.03-1.36) than in the GN groups (1.03, RI 0.88-1.21, $P=.038$, Mann-Whitney test) and control (1.07, RI 0.88-1.20) groups ($P=.017$, Mann-Whitney test).

In the CIN group 12 patients (9.38%; 95%CI: 5.44-15.67) had a cystatin-estimated GFR higher than 60 mL/min and a creatinine-estimated GFR <60 mL/min. In the GN group, the same conditions affect nine subject (6.38%; 95%CI: 3.94-11.54) (not significant, Chi-square test). Therefore, no significant changes in the classification were observed. In the same way, in the CIN group only eight patients (6.25%, 95%CI 3.20-11.85) have normal serum cystatin levels but a GRF lower than 60 mL/min.

4 | DISCUSSION

Patients with chronic interstitial nephropathy showed lower serum levels of cystatin C values defined as cystatin C/creatinine rate. Conversely, the quotient between cystatin C-estimated GFR and creatinine-estimated GFR was higher in this kind of patients when they were compared with glomerulonephritis patients and control groups without kidney disease. Nevertheless, these changes had minor effects on CKD patient's classification.

Cystatin C, which belongs to the type II cystatin gene family, is a non-glycosylated 13-kDa protein constitutively secreted shortly after its synthesis.⁸ Because its low-molecular mass and its positive charge at physiologic pH allow it to be freely filtered by kidney glomeruli and because it is normally reabsorbed and afterwards almost completely catabolized by proximal tubular cells.⁹ The cystatin C that is not metabolized is eliminated in urine and may represent a useful marker of tubular injury¹⁰ or renal tubular dysfunction.¹¹ Because no circadian rhythm is evident in healthy individuals, we can assume that if renal tubules are fully functional and catabolize the majority of freely filtered cystatin C, only small amounts of this marker would be found in urine. Hence, the presence of increased cystatin C concentrations in single-void urine samples would directly reflect tubular damage.¹²

In this regard, an automatic quantitative assay to measure CST3 concentrations in urine using a Behring N-Latex Cystatin C kit on a BNII laser nephelometer has been developed.⁵ Urinary cystatin C concentrations in glomerular disease patients were not statistically

different from those in control subjects. Conversely, mean urinary cystatin C concentrations in the patients with kidney tubular were significantly higher than in controls and glomerular disease patients. Thus, it has been suggested that urinary cystatin C levels could be relevant in discriminating tubulopathies. Later, it has been found conflicting results on the use of urinary cystatin C/urinary creatinine rate. These errors can be avoided when estimates of tubular damage are based on urinary cystatin C concentrations alone. It could be hypothesized that serum cystatin C levels could be lower in CIN patients because of a higher urinary cystatin excretion.

We have found a small but significant difference in the serum cystatin C/serum creatinine level from CIN patients to GN and control ones although cystatin C levels were higher in CIN patients. Nonetheless, it could be expected since CIN patients group had a worse renal dysfunction as measured by serum creatinine levels and creatinine-estimated GFR. This difference merits further attention. Two meta-analyses about the use of cystatin C as an endogenous marker of GFR in general populations and in CKD patients agreed that serum cystatin is superior to serum creatinine to rule in renal impairment in the cut-off range of GFR between 60 and 79 mL/min/1.73 m².^{13,14} The utility of cystatin C in predicting the clearance of drugs which were eliminated either exclusively or only partially by the kidneys has been demonstrated by studies. Interestingly, these studies showed an advantage of combining cystatin C with creatinine rather than using either individually.^{15,16} This suggests that the two parameters are not entirely redundant and that serum cystatin does not only depend on GFR.

The renal system carries several physiologic roles but GFR is considered the best surrogate of overall kidney function and, for this reason, its assessment has become an important tool in clinical practice. GFR cannot be measured directly, but instead can be estimated by the clearance of filtration markers.¹⁷ Regardless, the clinical assessment of GFR can aid the clinician in estimating the degree of renal dysfunction and/or progression of established kidney disease. Various creatinine-based equations have been developed in an attempt to improve the estimation of GFR from serum creatinine. The most widely used equations are the Cockcroft-Gault equation¹⁸ and the 4-variable Modification of Diet in Renal Disease (MDRD).¹⁹ Specifically, it is now recommended that all creatinine measurements are IDMS traceable and this traceability is of the highest importance in the context of creatinine-based equations.²⁰ Nevertheless, current KDIGO Guidelines recommend CKD-EPI equation.³ This last formula is the one used in our study though, in our experience the difference

of results between both equations is quite small.²¹ As it could be expected, from the results about serum cystatin C/serum creatinine levels, the index between cystatin C-estimated GFR and creatinine-estimated GFR was slightly higher in the CIN group than in the GN and control group. This could have produced the patient's misclassification but the risk of changes is very low in our sample.

Conceptually, glomerular diseases are diagnosed by high albumin urine excretion meanwhile heavy proteinuria is seldom found in CIN subjects. It has been reported in a small subset of pediatric patients that massive proteinuria can block reabsorption and further degradation of low-molecular weight proteins, such as cystatin C, in the tubules as cystatin C was detectable in the urine of the children.²² Therefore, decreased serum cystatin C levels could also be expected in glomerular disease patients, but we have not found this relationship. Contrarily, in a group of hypertensive patients with normal serum creatinine and GFR above 60 mL/min an increased serum cystatin C level was found in near 15% of the sample. In these subjects, the prevalence of microalbuminuria and overt proteinuria was increased when compared with those having lower cystatin C levels. These patients were older and also showed a lower GFR.²³ Therefore, cystatin C seems to be a better marker than serum creatinine, and more sensitive than the estimated GFR, for estimating CKD progression risk.

We can draw several conclusions: Patients with chronic interstitial nephropathy had lower serum levels of cystatin C values defined as cystatin C/creatinine rate when they were compared with glomerulonephritis affected subjects and control ones. In the same way, the index between cystatin C-estimated GFR and creatinine-estimated GFR was higher in this kind of patients. Nevertheless, these changes had minor effects on the clinical CKD patient's classification.

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