URINARY CYSTATIN C AS A MARKER OF TUBULAR DYSFUNCTION

CISTATIN C U URINU KAO POKAZATELJ TUBULSKOG OŠTEĆENJA

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Summary: Cystatin C (CysC) is a nonglycosylated 13 KD protein that belongs to the type II cystatin gene family. It is a strong inhibitor of cysteine proteinases, freely filtered by the kidney glomerulus and reabsorbed by the tubulus, where it is almost totally catabolized. Remainder of the nonmetabolized CysC is eliminated in urine and may represent a useful marker of tubular dysfunction. The aim of the study was to confirm the clinical importance of the quantitative determination of CysC by an automated immunonephelometric method (DADE Behring). Two groups of patients were examined: one with glomerular (GD, n=36) and one with tubular dysfunction (TD, n=31), and compared with the control group (CG, n=31) of healthy males and females from laboratory personnel (n=11) and patients on routine systematic examination (n=20), from 25 to 58 years old. The patient groups were categorised according to the urine analysis of total proteins, creatinine and adequate proteins electrophoretic panel. CysC concentration in CG was in the range of 0.02–0.15 mg/L; 0.01–0.48 mg/L and 0.25–18 mg/L in GD and TD groups respectively. Values of means ± SD for patient groups (GD=0.11± 0.14; TD=3.92 ± 3.75 mg/L) showed statistical significance ($p<0.001$) in the TD group in relation to GD and CG groups. It confirms that quantitative determination of CysC in one urine portion, with a fast laboratory method, might be a useful marker of tubular dysfunction, especially in emergency situations, taking into account that there is no interference of circadian variation on its concentration.

Keywords: cystatin C, kidney, tubular marker, tubulopathy, urine

Kratak sadržaj: Cistatin C (CysC) je neglikozirajući protein molekulske mase od 13 KD koji pripada tipu II genske familije cistatina. Ima funkciju inhibitora cistein proteinaze koji se potpuno filtrira u glomerulima a zatim reapsorbuje i kataboliše u proksimalnim tubulima. Preostali i nerazgrađen CysC biva eliminisan urinom pa zato može da bude koristan marker tubulskih oštećenja. Cilj ispitivanja bio je da se utvrdi klinički značaj kvantitativnog određivanja CysC automatizovanom imunonefelometrijskom metodom (DADE Behring). Ispitane su dve grupe bolesnika: sa tubulskom (TI) i glomerulskom (GI) insuficijencijom, i upoređene sa kontrolnom grupom (KG) koju su činile zdrave osobe oba pola (n=31), laboratorijski personal (n=11) i pacijenti na sistematskom pregledu (n = 20), starosne dobi od 25 do 58 godina. Kategorizacija grupa sa GI (n=36) i TI (n=31) ustanovljena je na osnovu koncentracije ukupnih proteina, kreatinina i adekvatnih elektroforetskih denzitometrijskih varijanti karakterističnih za obe grupe. Koncentracija CysC u KG bila je u opsegu 0,02–0,15 mg/L, u grupi sa GI 0,01–0,48 mg/L, a u grupi sa TI 0,25–18 mg/L. Rezultati vrednosti (medijana±SD) za grupu sa GI (0,11 ± 0,14 mg/L) i grupu sa TI (3,92 ± 3,75 mg/L) pokazali su statistički značajnu razliku (p< 0,0001) između grupe sa TI i grupa sa GI i KG. Pokazano je da određivanje CysC u jednom uzorku urina brzim laboratorijskim postupkom može biti koristan test za diferencijalnu dijagnostiku tubulskih u odnosu na glomerulska oštećenja, posebno u urgentnim stanjima, s obzirom na činjenicu da na njegovu koncentraciju nema uticaja cirkadijalnih ritmova.

Ključne reči: cistatin C, bubreg, tubulski marker, oštećenje tubula, urin

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Introduction

Cystatin C (Cys C) is a 13-kDa endogenous cysteine proteinase inhibitor produced by nucleated cells at a constant rate, freely filtered by the glomerulus and reabsorbed and catabolized, but not secreted by the tubules (1, 2). Serum Cys C has been previously

introduced as a new marker to estimate glomerular filtration rate (GFR) and has demonstrated higher diagnostic value than serum creatinine in detecting reduced GFR (3). In normal renal function, tubular reabsorption and catabolism of Cys C are almost complete and it is only detected in very small quantities in the urine. Increased urinary Cys C was noted especially in renal disorders such as the tubular damage associated with HIV nephropathy, and acute interstitial nephritis (4–6). It is a well-known inhibitor of cysteine proteinases (7). It should, therefore, exhibit a protective function by regulating the activities of endogenous cysteine proteinases that cause uncontrolled proteolysis and tissue damage. Cys C belongs to the type II cystatin gene family, and it is a nonglycosylated protein constitutively secreted shortly after its synthesis (8, 9). Based on its high concentration in biologic fluids, it is probably one of the most important extracellular inhibitors of cysteine proteinases (10–12).

Serum concentrations of Cys C appear to be independent of sex and muscular mass (13, 14), and are constant throughout life, from 1 to 65 years of age (0.6–1.0 mg/L). Its low molecular weight and positive charge at physiological pH mean that it is freely filtered by the kidney glomerulus. Thus, its plasma half-life in humans is about 2 hours. These properties, associated with a lack of tubular secretion, mean that Cys C is a suitable biological marker that is now being widely used in nephrology to assess glomerular filtration (15–20).

After glomerular filtration, Cys C is reabsorbed by the proximal tubular cells, where it is almost completely catabolized (21, 22), with the remaining uncatabolized Cys C eliminated in the urine. Therefore, normal urinary Cys C (u Cys C) concentrations are very low, as reported to be in the range of 0.03–0.3 mg/L (22). In the case of tubular diseases, it could be postulated that Cys C degradation would be reduced and, consequently, an increase in its urinary elimination would be observed. Thus, u Cys C might be a reliable marker of tubular dysfunction (TD). Therefore, the aim of the study was to confirm the clinical importance of the quantitative determination of u Cys C by a fast and automated immunonephelometric method in daily diagnostic procedure.

Patients and Methods

The examination was carried out using samples obtained during the normal clinical follow-up of patients, and two groups were examined: one with glomerular (GD, n=36) and another with tubular dysfunction (TD, n=31), which were compared with the control group (CG, n=31) of healthy males and females from laboratory personnel (n=11) and patients on routine systematic examination (n=20), from 25 to 58 years of age. The patient groups were cate-
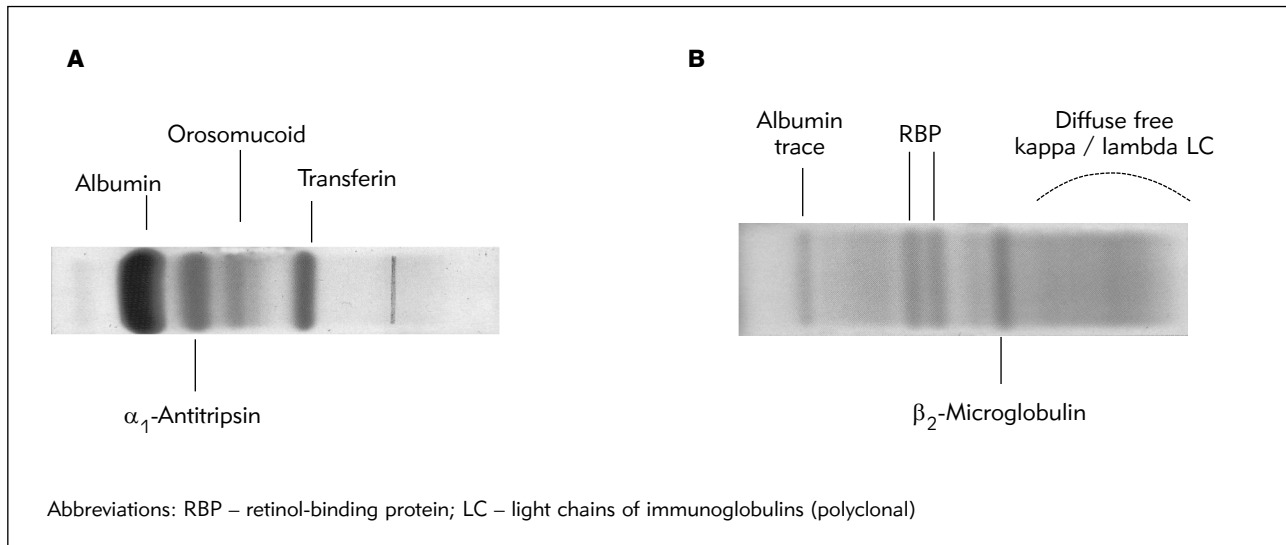
gorised according to the ordinary clinical findings, urinary analysis of total proteins, creatinine and adequate proteins electrophoretic panel. Urine samples were collected from patients in one portion and analyzed after centrifugation at 5000 rpm. The concentration of u Cys C was examined by the particle enhanced latex immunonephelometry (PENIA) method, on a BN II analyzer (DADE Behring). The kit was designed for Cys C serum analysis and has a limit of detection of 0.17 mg/L. In plasma or serum, two subsequent dilutions of the sample (1:5 and 1:20) are performed to obtain a 1:100 final dilution for the Cys C/anti-Cys C reaction. The analyzer program was slightly modified to allow the quantification of u Cys C, the usual concentration of which is ten-fold lower than in plasma. To increase sensitivity, we omitted the second sample dilution, which allows u Cys C detection at a concentration as low as 0.008 mg/L. The omission of this automated sample dilution step is the only modification made in the nephelometric program settings. Calibration and controls were performed using the same materials as for serum/plasma determination. Creatinine concentration was measured by the Jaffe kinetic method, urinary total proteins with the turbidimetric Benzethonium chloride procedure on a Hitachi 912 apparatus (Roche, Mannheim, Germany) according to the manufacturer's instructions. Urine electrophoresis was estimated on a Hydragel-Hydrasis (Sebia, France) automatic instrument. All statistical analyses were performed using Basic Statistic software for comparison of means (Mann-Whitney U test), standard deviation and data distribution.

Results

Urine electrophoresis for each individual patient sample was performed in order to confirm the possible type of proteinuria. Typical electrophoretic patterns for glomerular (a) and tubular (b) proteinuria are presented in *Figure 1*.

Two populations of patients with renal dysfunction were analyzed with similar age, gender and male/female ratio. A total of 31 patients with documented TD (serum creatinine 463 ± 279 μmol/L, mean ± SD) were compared to 36 patients with documented GD (mean serum creatinine 262 ± 155 μmol/L). The mean serum creatinine in controls was 78 ± 29 μmol/L (*Table I*).

Urinary Cys C concentrations in CG were within the range of 0.02–0.15 mg/L. Those values in the group with GD were 0.0–0.48 mg/L, but were much higher in the group with TD ranging between 0.25 and 18 mg/L. Obtained values of means ± SD for the group with TD (3.92 ± 3.75 mg/L) in relation to the group with GD (0.11 ± 0.14 mg/L) and the CG (0.07 ± 0.011 mg/L) showed statistical significance ($p < 0.0001$) by the Mann-Whitney U test (*Table I*). Values for u Cys C between CG and the group with GD did not differ ($p > 0.05$) statistically (*Figure 2*).

A

Albumin

Orosomucoid

Transferin

α_1-Antitripsin

B

Albumin trace

RBP

Diffuse free kappa / lambda LC

β_2-Microglobulin

Abbreviations: RBP – retinol-binding protein; LC – light chains of immunoglobulins (polyclonal)

Figure 1 Electrophoretic pattern presentation of A) glomerular proteinuria, B) tubular proteinuria.

Table I Demographic, creatininemia and cystatinuria data for the 67 patients and 31 healthy controls.

Parameter	CG	GD	TD
n	31	36	31
Age (mean±SD), years	44.1 ± 9.3	57.6 ± 12.6	56.1 ± 11.1
Sex, M/F	17/14	19/17	15/16
Creatininemia, μmol/L	78 ± 29	262 ± 149	463 ± 279
u Cys C, mg/L	0.07 ± 0.11	0.11 ± 0.14	3.92 ± 3.75*

Abbreviations: CG – control group; GD/TD – groups with glomerular and tubular dysfunction * – statistical significance ($p < 0.0001$).

Abbreviations: CG – control group; GD – group with glomerular dysfunction; TD – group with tubular dysfunction; * statistical significance $p > 0.05$; **– $p < 0.0001$
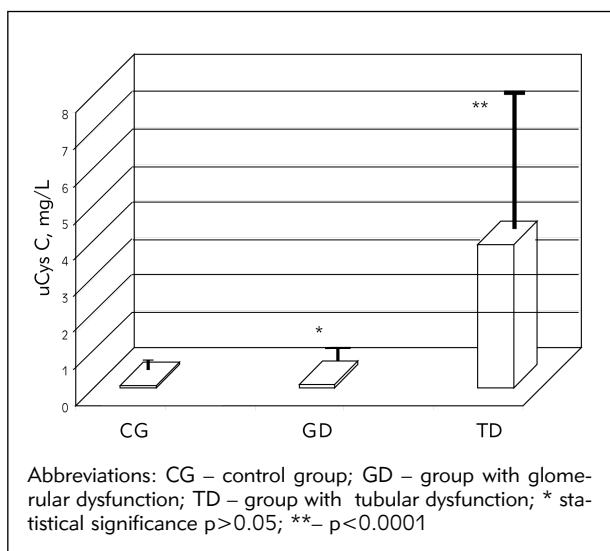
Figure 2 Statistical presentations of u Cys C concentrations among three tested groups.

Discussion

Glomerular proteinuria is due to increased filtration of macromolecules (such as albumin) across the glomerular capillary wall. Tubular proteinuria results from increased excretion of low molecular weight proteins (LMW), such as β_2-microglobulin (β_2-M), immunoglobulin light chains, retinol-binding protein (RBP), and amino acids. These molecules are normally filtered across the glomerulus and then largely reabsorbed in the proximal tubule. Interference with proximal tubular reabsorption, due to a variety of tubulointerstitial diseases, can lead to increased excretion of these smaller proteins. In spite of the mentioned LMW proteins as a marker of TD, u Cys C appeared as a relatively new urinary marker in the laboratory, distinguishing TD from GD. Cys C has advantages as a urinary marker of tubular injury compared to the established markers (23). In contrast to β_2-M, infection, inflammation and malignancy do not increase the urinary concentration of Cys C as it is produced and filtered at a constant rate (1, 2, 23, 24). In addition, it is more stable in the urine than α_1-microglobulin, β_2-M or RBP, and may have age and gender independent reference values (25, 26). Furthermore, Cys C demonstrated good stability in routine urine examinations and storage conditions (pH, temperature, plastic vials etc.). Neither grossly proteinuric, icteric, nor hemolytic samples affected measurement of Cys C concentrations in the urine (27). Our results, under similar measurement conditions, showed that concentrations in CG correspond well with the reference values previously published for sera (6, 13, 14) and urine (27). High statistical significance among group with TD vs. group with GD showed that u Cys C measurement could be an excellent and fast laboratory method for detecting tubular insufficiency, much more accurate than the other

mentioned LMW proteins. In a recent study (23), it was demonstrated that the urinary excretion of Cys C accurately predicts the requirement of renal replacement therapy in acute tubular necrosis (ATN). This is important, as ATN requiring renal replacement therapy is associated with a high mortality (28). Therefore, determination of cystatin C may help to intensify early supportive care or to initiate therapeutic interventions that may consequently improve the outcome of ATN.

In conclusion, the measurement of Cys C in the urine by PENIA achieved a good performance. This method offers rapid, automated and precise measurement of cystatin C in comparison to earlier methods, which were slower, semi-quantitative, less precise, or not commercially available (5, 6, 29). Additional benefits are the high stability of urinary Cys C, the lack of interference and the lack of variability with the method of urine collection, age and gender. These features are particularly important when urinary Cys C is used as a routine biochemical test of acute tubular injury (30) in clinical practice, especially in emergency laboratory diagnostics in critically ill patients.

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