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Original paper

INFLUENCE OF PROTEINURIA ON CYSTATIN C SERUM CONCENTRATION IN PATIENTS WITH PRIMARY GLOMERULONEPHRITIS

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Summary: Cystatin C is a low molecular weight protein which can be used as a marker of glomerular filtration rate (GFR). By comparing GFR (determined with ^{51}Cr-EDTA) of cystatin C and creatinine, cystatin C was found to be superior to creatinine as an indicator of GFR since the serum creatinine concentration changes significantly only when GFR is already impaired by 50%. Cystatin C serum concentration is mainly determined by GFR. Combined measurement of cystatin C serum concentration and its excretion in urine is useful in assessing early decrease of GFR and proximal tubule damage. The aim of this study was to assess the influence of proteinuria on cystatin C serum concentration. We determined the serum cystatin C concentration, 24h protein excretion in urine, serum urea, creatinine, total proteins and albumin concentration, plasma colloid osmotic pressure (COP) and endogenous creatinine clearance. The study included 45 patients (28 males and 17 females), average age 39.51 ± 7.60 years, with average endogenous creatinine clearance 97.70 ± 12.08 mL/min. Patients were divided into three groups according to the level of glomerular proteinuria. The first, control group, included 15 persons (6 males and 9 females, average age 37.27 ± 5.48 years) with proteinuria level <0.25 g/24h and average endogenous creatinine clearance 99.70 ± 12.94 mL/min. Patients with primary glomerulonephritis (PGN) in the second group (11 males and 4 females, average age 38.93 ± 8.32 years) had proteinuria range 0.25–3.5 g/24h and endogenous creatinine clearance 94.00 ± 11.10 mL /min. The third group of patients with nephrotic proteinuria (>3.5 g/24h) included 15 patients (11 males and 4 females, average age 42.33–8.23) with PGN and endogenous creatinine clearance 99.40–12.07 mL/min. Results were statistically analysed with Student t-test and Mann-Whitney test, with levels of significance 0.05 and 0.01. Proteinuria had no significant influence on cystatin C serum concentration in patients with primary glomerulonephritis and endogenous creatinine clearance over 80 mL/min. Cystatin C is an adequate indicator of glomerular filtration rate in proteinuric patients.

Key words: cystatin C, creatinine, glomerular filtration rate, proteinuria

Introduction

Renal function in clinical nephrology can be assessed by serum creatinine concentration, endogenous creatinine clearance, inulin clearance (the »golden standard«), clearance of various radionuclid markers (^{99m}Tc-DTPA) and low molecular weight plasma proteins (α_1-microglobuline, β_2-microglobuline, retinol-binding protein). The simplest and most widely used parameter for assessment of renal function is serum creatinine concentration. However, serum creatinine increases only when glomerular filtration rate (GFR) falls to about 60 mL/min (1, 2). According to NCCLS (*National Committee for Clinical Laboratory Standards*) guidelines, normal serum creatinine values range from 57 to 95 mmol/L for females, and from 69 to 111 mmol/L for males (3). Linear decrease of reciprocal serum creatinine value during a certain period of time (so called 1/Cr curve) is used

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for assessment of progression rate of chronic renal failure. The time period during which creatinine serum concentration doubles is used as a parameter for assessing progression of chronic renal failure and efficacy of applied therapy (2). In hypertensive patients or those with family history of renal diseases and pathological findings in urine, or with serum creatinine concentration above normal values, GFR should be determined. Endogenous creatinine clearance and clearance of radionuclide isotopes are used for the assessment of GFR. The golden standard for determining GFR is clearance of inulin and other markers, such as ^{125}I – iothalamate (4–6).

Cystatin C serum concentration can also be used for the assessment of GFR. Cystatin C is a plasma protein with molecular weight of 13 kD. Due to its low molecular weight, it is easily filtered through glomerular basement membrane, and more than 99% is reabsorbed in epithelial cells of proximal tubules. Therefore, because of its constant rate of production by nucleated cells, its serum concentration is determined by glomerular filtration, which makes it a good marker of GFR (7–15). There is no statistically significant difference between serum cystatin C concentration in men and women. Normal cystatin C values, as suggested by NCCLS (National Committee for Clinical Laboratory Standards) range from 0.54 to 1.21 mg/L (3). It is determined by immunonephelometric method. Combined measurement of cystatin C serum concentration and its urine excretion is useful in the assessment of early decrease of GFR and proximal tubules damage (17, 18). Daily urine excretion of cystatin C is 0.0074 ± 0.0034 mg. Very low cystatin C concentration in urine (< 0.3 mg/L) limits its routine use in assessment of tubulointerstitial damage.

The aim of this study was to determine the influence of various proteinuria levels on cystatin C serum concentration.

Patients and Methods

The study included 45 patients treated at the Institute of Urology and Nephrology, Clinical Center of Serbia in Belgrade, over a period of one year. All subjects gave informed consent to participate in the study, according to Helsinki Declaration on Medical Research.

Patients included in the study had primary glomerular diseases, endogenous creatinine clearance > 80 mL/min and various levels of glomerular proteinuria. Urinary infection was excluded by microscopic and microbiologic examination of each urine sample.

Patients were divided into three groups based on proteinuria levels: group I (control) with proteinuria < 0.25 g/24h, group II (nonnephrotic proteinuria) with proteinuria 0.25–3.5 g/24h and group III (nephrotic proteinuria) with proteinuria > 3.5 g/24h (*Table I*).

Blood and urine samples were taken in the morning. Twenty-four hour urine was collected from 6 A.M. one day to 6 A.M. the next day. Creatinine

Table I General patients' data

General data		Groups (24h-proteinuria)		
		I (< 0.25 g/24h)	II (0.25–3.5 g/24h)	III (≥ 3.5 g/24h)
		Mean ± SD	Mean ± SD	Mean ± SD
Number (N)		15	15	15
Sex (m/f)		6/9	11/4	11/4
Age (years)		37.27 ± 5.48	38.93 ± 8.32	42.33 ± 8.23
CCr (mL/min)		99.70 ± 12.94	94.00 ± 11.10	99.40 ± 12.07
Healthy subjects		15	/	/
Primary GN	MsGN	/	9	/
	MGN	/	/	7
	MPGN	/	/	2
	GNMP	/	3	3
	FSGS	/	3	3

N – number of subjects, m – male, f – female, SD – standard deviation, Ccr – endogenous creatinine clearance, PGN – primary glomerulonephritis, MsGN – mesangioproliferative glomerulonephritis, MGN – membranous glomerulonephritis, MPGN – membranoproliferative glomerulonephritis, GNMP – minimal change glomerulonephritis, FSGS – focal segmental glomerulosclerosis

concentration in serum was determined by colorimetric test, on Monarch plus IL, Milan, Italy, apparatus. Creatinine concentration in 24h urine was determined in the same way with ten times higher dilution. Urea serum concentration was determined by enzyme method (urease-glutamat dehydrogenase) on the same apparatus.

Proteinuria in samples of 24h urine was determined by colorimetric test with CBB. Cystatin C serum concentration was determined by PENIA method (*Particle-Enhanced Nephelometric Immuno-Assay*) from Dade-Behring Co. The reference range for cystatin C serum concentration is 0.54–1.21 mg/L. Coloid-osmotic pressure (COP) was calculated from the equation (19):

$$COP\ (mmHg) = \alpha(P\text{-}Alb)^2 + \beta[Alb \times (P\text{-}Alb)]$$

proteinuria 3.5 g/24h a = 0.13 i b = 2.07

proteinuria < 3.5 g/24h a = –1.15 i b = 3.03,

where P stands for plasma total protein concentration (g/L) and Alb represents plasma albumin concentration (g/L). Normal COP ranges from 24 to 26 mmHg.

Group differences were tested by the Student t-test and Mann-Whitney U test.

Results

In order to assess renal function, we determined urea, creatinine and cystatin C serum concentrations, proteinuria and endogenous creatinine clearance in patients with primary glomerular diseases. Results are shown in *Table II*.

No statistically significant differences were found between groups concerning serum urea and creatinine, and endogenous creatinine clearance values.

Patients with proteinuria >3.5 g/24h had significantly lower total proteins and albumin serum concentrations ($p<0.01$) than patients with proteinuria range 0.25–3.5 g/24h (*Table II*). Between other patients' groups, no statistically significant differences were found concerning serum albumin and total proteins concentration ($p>0.05$).

Patients with proteinuria > 3.5 g/24h had significantly lower COP ($p<0.01$) than control subjects and patients with nonnephrotic proteinuria. No statistically significant difference in COP was found between other groups ($p>0.05$).

The mean values of cystatin C in examined groups were normal, but higher in patients with proteinuria. All parameters are presented in *Tables I* and *II*.

No statistically significant difference in cystatin C serum values ($p>0.05$) was found between groups.

Discussion

Serum cystatin C concentration was used to estimate renal function, as it is a better marker of GFR decrease than serum creatinine and clearance of endogenous creatinine.

By investigating the correlation between GFR (determined by clearance of ^{51}Cr-EDTA), serum cystatin C and serum creatinine, it was found that cystatin C is a better marker for GFR than serum creatinine concentration (20). By investigating correlations between ^{99m}Tc-DTPA clearance and 1/cys C, 1/creatinine and endogenous creatinine clearance (CCr), it was found that correlation between ^{99m}Tc-DTPA clearance and 1/cys C was the best (17, 18). In patients with normal and mild renal insufficiency (^{99m}Tc-

Table II Examined parameters according to level of glomerular filtration

Parameters	Endogenous creatinine clearance (mL/min)		
	< 0.25 g/24h (I)	0.25–3.5 g/24h (II)	≥ 3.5 g/24h (III)
	Mean ± SD	Mean ± SD	Mean ± SD
Total proteins (g/L)	70.00 ± 3.57*	68.33 ± 4.76**	51.87 ± 6.46
Albumines (g/L)	42.93 ± 3.61*	41.87 ± 4.88**	25.67 ± 4.06
COP (mmHg)	26.44 ± 2.71*	24.72 ± 3.47**	14.87 ± 3.75
Urea (mmol/L)	5.03 ± 0.92*	5.61 ± 1.27	5.86 ± 1.64
Creatinine (μmol/L)	85.34 ± 13.60*	92.40 ± 11.33	90.00 ± 26.92
Creatinine clearance (mL/min)	99.70 ± 12.94*	94.00 ± 11.10	99.40 ± 12.07
Cystatin C (mg/L)	0.82 ± 0.20*	1.00 ± 0.42	1.07 ± 0.39
Significance	♦ p I,III < 0.01	♦♦ p II,III < 0.01	

N – number of subjects, SD – standard deviation, COP – plasma colloid-osmotic pressure

DTPA clearance 50–80 mL/min) cystatin C is a liable marker of GFR (17, 18, 20). Assessment of GFR by radionuclid clearance is not safe to perform in patients with progressive renal diseases and GFR values lower than 20–30 mL/min (10).

Combined measurement of cystatin C concentration in serum and in urine is valuable for assessing early decrease of GFR and damage of proximal tubules (21). Proteinuria has no statistically significant influence on cystatin C serum concentration in patients with endogenous creatinine clearance > 80 mL/min. Patients with endogenous creatinine clearance < 30 mL/min excrete significantly higher amounts of cystatin C in urine than subjects with normal endogenous creatinine clearance values. Increase of fraction cystatin C clearance represents deterioration of proximal tubule epithelial cells function (7).

Renal function gradualy decreases in patients with chronic renal diseases. Measuring creatinine serum concentration as the marker for GFR should be performed at least once a year in patients with chronic renal diseases, and even more often in patients with GFR < 60 mL/min/1.73 m^2, rapid decrease of GFR (≥ 4 mL/min/1.73 m^2/year), risk factors that contribute to rapid decrease of renal function, during treatment for slowing down the progression of renal disease, and during exposure to risk factors for acute decrease of GFR (4, 5). In patients with chronic kidney diseases, renal function decreases progressively, irrespective of cessation of primary event that caused the disease. In patients with GFR < 60 mL/min/1.73 m^2 end-stage renal failure (GFR < 15 mL/min/1.73 m^2) develops in 10 years. Decrease rate of renal function is ≥ 4 mL/min/1.73 m^2/year (4, 5).

Proteinuria has no statistically significant influence on cystatin C serum concentration in patients with primary glomerulonephritis and endogenous creatinine clearance over 80 mL/min.

UTICAJ PROTEINURIJE NA KONCENTRACIJU CISTATINA C U SERUMU KOD BOLESNIKA SA PRIMARNIM GLOMERULONEFRITISOM

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Kratak sadržaj: Cistatin C je protein male molekulske mase koji može da se koristi za procenu jačine glomerulske filtracije. Ispitivanjem stepena povezanosti između JGF (određene pomoću ^{51}Cr-EDTA klirensa), *cistatina C* i kreatinina u serumu, utvrđeno je da je *cistatin C* bolji pokazatelj JGF u odnosu na koncentraciju kreatinina u serumu jer je poznato da se 50% JGF izgubi pre nego što dođe do značajnije promene u koncentraciji serumskog kreatinina. Koncentracija cistatina C u serumu uglavnom zavisi od jačine glomerulske filtracije. Kombinovano merenje koncentracije *cistatina C* u serumu i količine izlučivanja *cistatina C* mokraćom, korisno je za procenu početnog smanjenja jačine glomerulske filtracije i oštećenja proksimalnih tubula bubrega. Zbog toga je cilj ovog rada bio da se ispita uticaj proteinurije na koncentraciju cistatina C u serumu. Pored određivanja koncentracija cistatina C u serumu i proteina u 24h urinu, određene su i koncentracija uree, kreatinina, ukupnih proteina i albumina u serumu, koloidno-osmotski pritisak plazme (COP) i klirens endogenog kreatinina. Ispitano je 45 bolesnika (28 muškaraca i 17 žena), prosečne starosti 39,51 ± 7,60 godina, sa prosečnim klirensom endogenog kreatinina 97,70 ± 12,08 mL/min. Zavisno od stepena glomerulske preteinurije ispitanici su podeljeni u tri grupe. U prvoj grupi sa proteinurijom < 0,25 g/24h (kontrolna grupa) bilo je 15 osoba (6 muškaraca i 9 žena), prosečne starosti 37,27 ± 5,48 godina, s prosečnim klirensom endogenog kreatinina 99,70 ± 12,94 mL/min. Druga grupa sa proteinurijom nenefrotskog ranga od 0,25 do 3,5 g/24h imala je 15 bolesnika (11 muškaraca i 4 žene) sa PGN, prosečne starosti 38,93 ± 8,32 godina, s prosečnim klirensom endogenog kreatinina 94,00 ± 11,10 mL/min. Treću grupu sa proteinurijom nefrotskog ranga >3,5 g/24h činilo je 15 bolesnika (11 muškaraca i 4 žene) koji boluju od PGN, prosečne starosti 42,33 ± 8,23 godina, s prosečnim klirensom endogenog kreatinina 99,40 ± 12,07 mL/min. Za statističku analizu dobijenih podataka korišćeni su Student-ov T-test i Mann Whitney-ev U-test. Prag značajnosti bila je verovatnoća od 0,05 i 0,01. Utvrđeno je da proteinurija nema statistički značajnog uticaja na koncentraciju cistatina C u serumu kod bolesnika sa primarnim glomerulonefritisom i klirensom endogenog kreatinina > 80 mL/min tako da je cistatin C stabilan parametar za procenu JGF i u stanjima proteinurija.

Ključne reči: cistatin C, kreatinin, glomerulske filtracije, proteinurija

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