CYSTATIN C IN THE DIAGNOSIS OF CHRONIC KIDNEY DISEASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

UDC [616.61–002.2+616.379–008.64]–07
Received 13.05.2013

© **N.A. Yarkova,** PhD, Tutor, the Department of Hospital Therapy named after V.G. Vogralik;
N.N. Borovkov, D.Med.Sc., Professor, the Head of the Department of Hospital Therapy named after V.G. Vogralik;
O.V. Zanozina, D.Med.Sc., Professor, the Department of Hospital Therapy named after V.G. Vogralik;
V.P. Nosov, D.Med.Sc., Professor, the Department of Hospital Therapy named after V.G. Vogralik

Nizhny Novgorod State Medical Academy, Minin and Pozharsky Square, 10/1, Nizhny Novgorod, Russian Federation, 603000

The aim of the investigation was to estimate the role of cystatin C in the diagnosis of chronic kidney disease in patients with type 2 diabetes mellitus (DM2).

Materials and Methods. The study involved 97 patients with DM2, their mean age being 57 [53; 63] years, DM2 duration — 7.7 [2.5; 12] years. In all patients we identified cystatin C level and calculated glomerular filtration rate using different methods.

Results. Cystatin C is a reliable marker of glomerular filtration rate in DM2 patients. It enables to diagnose chronic kidney disease in normal albumin excreted with urine, i.e. at early stages of diabetic nephropathy.

The determination of this value by cystatin C in boundary glomerular filtration rate level of under 60 ml/min/1.73 m^2 is more accurate than that calculated by Cockcroft–Gault and MDRD formulae.

Key words: cystatin C; chronic kidney disease; type 2 diabetes mellitus.

Cystatin C is a nonglycated protein with molecular weight of 13.4 kDa and isoelectric point at pH=9.3, belongs to the family of cysteine proteinase inhibitors and identical to post-gamma-globulin. It was first identified in patients with renal failure as a protein of cerebrospinal fluid and urine [1]. This protein is synthesized at a constant speed by all cells containing nuclei; freely filtrated through glomerular membrane; metabolized completely in kidneys; not secreted by proximal renal tubules [2–4]. According to numerous studies [5–15], maintaining normal serum level of cystatin C is due to a constant synthesis rate independent of age, sex, body mass, and its constant clearance rate, which is determined mainly by renal functions. In case of pathology its level in blood increases. The more severe the renal pathology, the worse cystatin C filtrates in kidneys and the higher its blood level is.

The main diagnostic criterion of chronic kidney disease (CKD) is glomerular filtration rate (GFR). Currently, cystatin C is recognized as the most accurate endogenous GFR marker (surpasses creatinine) [16–21]. Single measurement of its concentration in blood enables to calculate GFR more accurately [8, 9, 22, 23]. Cystatin C role in diabetic nephropathy has been studied by many researchers [24, 25], however its role in CKD diagnosis in type 2 diabetes mellitus (DM2) patients still remain underinvestigated.

The aim of the investigation was to estimate the role of cystatin C in the diagnosis of chronic kidney disease in patients with type 2 diabetes mellitus.

Materials and Methods. The study involved 97 patients (34 male and 63 female) with DM2 aged 57 [53; 63] years, admitted to neuroendocrinological department of N.A. Semashko Nizhny Novgorod Regional Clinical Hospital (Russia) over the period of 2010–2013. DM2 duration was 7,7 [2.5; 12] years. According to past histories, DM2 was first diagnosed in 30 patients. DM2 and carbohydrate metabolism compensation degree were determined in accordance with National standards for diabetes mellitus diagnosis and management, we determined CKD stages depending on GFR according to the recommendations of National Kidney Foundation, USA (2002) [26–30]. The study complies with the Declaration of Helsinki (adopted in Helsinki, Finland, June, 1964, and revised in October, 2000, Edinburg, Scotland) and was performed following approval by the Ethic committee of Nizhny Novgorod State Medical Academy (Russia). Written informed consent was obtained from every patient.

All patients underwent clinical, laboratory and instrumental examination. Glycosulated hemoglobin HbA1c was studied on analyzer D-10 with standard kits (BIO-RAD, France) using high performance liquid ion-exchange chromatography. Lipid spectrum indices were determined using diagnostic systems LLC “Olvex Diagnosticum” (Saint Petersburg, Russia). Creatinine level was studied in venous blood plasma according to a technique based on Jaffe reaction using diagnostic systems LLC “Olvex Diagnosticum”, urea level — using a diagnostic kit Diacom N

For contacts: Yarkova Nayaliya Alexandrovna, phone: +7 951-913-65-51; e-mail: n.a.yarkova@yandex.ru

on analyzer StatFax (Russia), and uric acid level — by color reaction on Konelab 60i (Finland).

We determined cystatin C in blood serum by immunoturbidimetric test using a diagnostic kit DiaSys (Germany). Normal values are 0.58–1.02 mg/ml. To assess renal filtration function we calculated GFR according to the formula suggested by Hoek et al. (2003): GFR [ml/min/1.73 m^2]=(80.35/cystatin C [mg/ml])–4.32. Alongside with that we calculated GFR using standard formulae of Cockcroft–Gault and MDRD [8, 9, 30].

To verify diabetic nephropathy diagnosis we tested 24-hour urine for microalbuminuria (MAU) by turbometric method on automatic analyzer Chem Well using a diagnostic kit Microalbumin (USA). An index under 30 mg/d was considered normal. The result from 30 to 300 mg/d was considered MAU. In case of a negative MAU result, we determined protein content in 24-hour urine.

To specify the presence of chronic kidney diseases of different etiology in DM2 patients we ran Addis–Kakovsky test, Zimnitsky's test, and microflora analysis. Nephrosonography was performed on MySono U5 (Medison, Korea) with obligatory measurement of kidney parenchyma size, the presence of cysts and calculi, the specification of calices-pelvis system anomalies. Ultrasonic Doppler examination with color flow mapping was performed on scanner PHILIPS-HD 11 XE (USA) to rule out renal artery stenoses, venous hypertension. All patients were examined by an experienced ophthalmologist and neurologist.

The findings of the investigation were statistically processed using Statistica 7.0. Analyzing the data we used nonparametric statistic methods in the form of median and 25 and 75 percentiles (Me [25p; 75p]), the reliability of one-factor differences of independent groups was determined using ANOVA according to Kruskal–Wallis test. Correlation relationships were analyzed using Spearman correlation coefficient with obligatory visual control of scattering diagrams and emission exclusion. P-values of <0.05 were considered statistically significant.

Results and Discussion. In the present study, while examining the patients and studying their medical records, the patients were found to have the following causes of CKD development (Table 1).

Table 1

Etiology of chronic kidney disease in DM2

Nosology	Number of patients with this pathology (n=97)	
	Abs. number	Percentage
Diabetic nephropathy	64	65.9
Ischemic nephropathy (renal artery stenosis)	1	1.0
Hypertensive nephropathy (renal tissue involvement due to arterial hypertension):	93	95.8
essential arterial hypertension	67	69
secondary arterial hypertension	26	26.8
Toxic nephropathy (toxic damage due to the action of drugs or contrast media)*	2	2.0
Urinary infection: chronic pyelonephritis	47	48.4
Urolithiasis	21	21.6
Multicystic kidneys	9	9.2
Calices-pelvis system anomalies	7	7.2
Hyperuricemia	38	39.1

* — toxic action due to uncontrolled long-term non-steroid anti-inflammatory drug intake due to frequent exacerbations of chronic musculoskeletal diseases.

The table demonstrating the causes of CKD in DM2 shows diabetic nephropathy to prevail among them (65.9%), and along with it, arterial hypertension and chronic pyelonephritis (95.8 and 48.4%, respectively) contributing to CKD. In 67 of 93 patients CKD was caused by essential arterial hypertension confirmed anamnestically and documentarily. Past history data and clinical and laboratory findings showed 48.4% of patients to have chronic pyelonephritis, predominantly latent. Purine metabolism disorders and urolithiasis (39.1 and 21.6%, respectively) also made their contribution to CKD, with hyperuricemia having no signs of urarthritis and tophi. Nephrolithiasis was diagnosed mainly in one kidney in 19 of 21 patients. CKD in DM2 patients rarely resulted from multicystic kidneys (9.2%) and calices-pelvis system anomalies (7.2%).

Thus, along with diabetic nephropathy, a significant contribution to CKD in DM2 was made by essential arterial hypertension, chronic pyelonephritis, hyperuricemia and asymptomatic urolithiasis.

Taking into account the incidence of diabetic nephropathy in CKD in DM2 it seemed obvious to represent clinical and laboratory findings of the patients depending on the stage of the disease (Table 2).

It should be emphasized that patients with diabetic nephropathy and normoalbuminuria were comparable by initial glycosulated hemoglobin level, body mass index, and total cholesterol.

Statistically significant differences between the groups of patients with normoalbuminuria and recorded diabetic nephropathy were found by the following indices: 24-h microalbumin excretion, the content of triglycerides, uric acid, urea, creatinine, cystatin C and GFR ($p<0.05$). The groups also had statistically significant difference in systolic and diastolic AP that only goes to show arterial hypertension to be one of leading factors of progressive diabetic nephropathy [15, 25, 26, 29].

According to ultrasound investigation, increased renal blood flow indices and moderate parenchyma changes were observed in two of 97 patients. These two patients had one kidney (another had been excised due to urolithiasis). Renal blood flow was generally all through seen, symmetrical, unaltered, indices of blood flow in renal arteries — within normal limits, renal vein were patent, with phase, symmetric blood flow, synchronous with breathing. Intima-media complex was not thickened.

The study of kidney filtration function calculated using cystatin C, GFR <60 ml/min/1.73 m^2 was revealed in 12 of 29 patients with normal albumin excreted with urine, in 17 of 43 patients — at the stage of microalbuminuria, in 4 of 11 patients — at the stage of proteinuria, in contrast to the calculation according to conventional Cockcroft–Gault and MDRD formulae (Table 3).

Table 2

Clinical and laboratory indices of patients with DM2 depending on diabetic nephropathy stage (Me [25p; 75p])

Index	Normoalbuminuria (n=35)	Microalbuminuria (n=46)	Proteinuria (n=11)	Chronic renal failure (n=5)	p
HbA1c, %	8.4 [6.9; 10]	8.7 [7.3; 10.2]	8.7 [7.5; 9.8]	8.9 [7.6; 10.3]	0.7
Microalbuminuria, mg/d	15.46 [12.1; 18.9]	70.20 [35.8; 88.2]	416.04 [364.8; 509.3]	—	0.01
Systolic blood pressure, mm Hg	140.7 [120; 150]	146.5 [130; 160]	157.0 [150; 165]	152.5 [145; 160]	0.04
Diastolic blood pressure, mm Hg	86.9 [80; 90]	89.6 [80; 100]	96.0 [90; 100]	96.2 [86; 100]	0.04
Body mass index, kg/m^2	33.2 [29.8; 35.0]	32.7 [30.1; 35.3]	34.7 [34.6; 36.9]	30.5 [27.4; 32.6]	0.07
Total cholesterol, mmol/L	5.04 [4.3; 5.9]	5.50 [4.8; 6.2]	6.40 [5.7; 7.1]	5.50 [4.8; 6.2]	0.1
Триглицериды, mmol/L	2.27 [1.2; 2.9]	2.48 [1.2; 3.0]	3.02 [2.3; 3.3]	3.00 [1.5; 4.5]	0.06
Urea, mmol/L	5.5 [4.5; 6.4]	6.8 [5.2; 7.9]	11.1 [8.0; 10.2]	16.5 [15.1; 17.9]	0.02
Creatinine, μmol/L	75.9 [63; 87]	79.8 [57; 92]	132.6 [92; 173]	223.5 [215.5; 231.5]	0.01
Uric acid, μmol/L	308.80 [246; 390]	334.94 [245; 360]	398.60 [342; 439]	439.60 [365; 519]	0.04
Cystatin C, mg/ml	1.12 [0.95; 1.29]	1.29 [0.92; 1.39]	1.8 [1.3; 2.3]	3.01 [2.9; 3.1]	0.01
GFR according to Cockcroft–Gault, ml/min	123.87 [91; 143]	116.50 [83; 150]	87.30 [65; 125]	33.20 [25.5; 40.5]	0.02
GFR according to MDRD, ml/min/1.73 m^2	87.60 [69; 106]	91.46 [64; 121]	55.10 [40; 70]	22.70 [20; 25.5]	0.04
GFR according to cystatin C, ml/min/1.73 m^2	69.60 [57.9; 80.2]	65.86 [53.4; 83]	56.8 [47.5; 72.6]	22.36 [21.3; 23.3]	0.04

GFR level below 60 ml/min/1.73 m^2 is chosen as threshold for a reason: it indicates even 50% loss of kidney filterability [13, 24, 28, 30]. GFR indices below 60 ml/min/1.73 m^2 indicate the danger of rapid progression of both renal, and related cardiovascular pathology [25, 27, 30].

When detecting the relationship between cystatin C level and other laboratory indices, we found the following positive correlations: with urea (r=0.54; p=0.001), creatinine (r=0.42; p=0.003), uric acid (r=0.39; p=0.002). Weak correlation relationship (statistically insignificant) were found between cystatin C and patients' age (r=0.18; p=0.05) and body mass index (r=0.11; p=0.05), microalbuminuria (r=0.13; p=0.06). Thus, cystatin C level does not depend on anthropometric indices, age and microalbuminuria.

Table 3

Glomerular filtration rate level depending on diabetic nephropathy stage

Diabetic nephropathy stage	GFR according to Cockcroft–Gault, ml/min		GFR according to MDRD, ml/min/1.73 m^2		GFR according to cystatin C, ml/min/1.73 m^2	
	<90	<60	<90	<60	<90	<60
Normoalbuminuria n=35	11	—	20	6	29	12
Microalbuminuria, n=46	17	5	22	7	43	17
Proteinuria, n=11	7	1	11	4	11	4
Chronic renal failure, n=5	—	5	—	5	—	5

Conclusion. The development of chronic kidney disease in type 2 diabetes mellitus is contributed by both diabetic nephropathy, and also other factors: essential arterial hypertension, latent chronic pyelonephritis, hyperuricemia and asymptomatic urolithiasis. Cystatin C is a reliable marker of glomerular filtration rate in such patients; it enables to diagnose chronic kidney disease in normal albumin excreted with urine, i.e. at early stages of diabetic nephropathy. The determination of this value by cystatin C in boundary glomerular filtration rate level of under 60 ml/min/1.73 m^2 is more accurate than that calculated by Cockcroft–Gault and MDRD formulae.

Study Funding. The study was financed by the authors.

Conflict of Interests. The authors have no conflict of interests to disclose.

References

1. Tonelle C.A, Edwards R.G., Stowe H.J., et al. Partial amino acid sequence of two forms of human post-gamma globulin. *Biochemical Biophysical Research Communications* 1979; 86(3): 613–619.

2. Rawlings N.D., Barrett A.J. Evolution of proteins of the cystatin superfamily. *Journal of Molecular Evolution* 1990; 30: 60–71.

3. Hall A., et al. Structural basis for the biological specificity of cystatin C. *Journal of Biological Chemistry* 1995; 270: 5115–5121.

4. Brown W.M., Dziegielewska K.M. Friends and relations of the cystatin superfamily — new members and their evolution. *Protein Science* 1997; 6: 5–12.

5. Chudleigh R.A., Ollerton R.L., Dunseath G., et al. Use of cystatin C-based estimations of glomerular filtration rate in patients with type 2 diabetes. *Diabetologia* 2009; 52: 1274–1278.

6. Newman D.J., Thakkar H., Edwards R.G., et al. Serum cystatin C measured by automated immunoassay: a more sensitive marker of changes in GFR than serum creatinine for glomerular filtration rate. *Kidney Int* 1995; 47: 312–318.

7. Shlipak M.G. Cystatin C as a marker of glomerular filtration rate in chronic kidney disease: influence of body composition. *Nat Clin Pract Nephrol* 2007; 3(4): 188–189.

8. Vervoort G., Willems H.L., Wetzels J.F. Assessment of glomerular filtration rate in healthy subjects and normoalbuminuric

diabetic patients: validity of a new (MDRD) prediction equation. *Nephrol Dial Transplant* 2002; 17: 1909–1913.

9. Pierrat A., Gravier E., Saunders C., et al. Predicting GFR in children and adults: a comparison of the Cockcroft–Gault, Schwartz and Modification of Diet in Renal Disease formulas. *Kidney Int* 2003; 64: 1425–1436.

10. Xie D., Joffe M.M., Brunelli S.M., et al. A comparison of change in measured and estimated glomerular filtration rate in patients with non-diabetic kidney disease. *Clin J Am Soc Nephrol* 2008; 3: 1332–1338.

11. Riordan S.E., Webb M.C., Stowe H.J., et al. Cystatin C improves the detection of mild renal dysfunction in older patients. *Ann Clin Biochem* 2003; 40: 648–655.

12. Fontsere N., Esteve V., Saurina A., et al. The search for a new marker of renal function in older patients with chronic kidney disease stages 3–4: usefulness of cystatin C-based equations. *Nephron Clin Pract* 2009; 112(3): 164–170.

13. Hojs R., Bevc S., Ekart R., et al. Serum cystatin C-based equation compared to serum creatinine-based equations for estimation of glomerular filtration rate in patients with chronic kidney disease. *Clin Nephrol* 2008; 70(1): 10–17.

14. Kravaritou M., Thanopoulou A., Karamanos B., et al. Evidence that even "normal" albuminuria may denote incipient GFR reduction in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2009; 85(3): 317–321.

15. Watanabe S., Okura T., Liu J., et al. Serum cystatin C level is a marker of end-organ damage in patients with essential hypertension. *Hypertens Res* 2009; 26: 895–899.

16. Grubb A., Bjork J., Lindstrom V., et al. A cystatin C-based formula without anthropometric variables estimates glomerular filtration rate better than creatinine clearance using the Cockcroft–Gault formula. *Scand J Clin Lab Invest* 2005; 65(2): 153–162.

17. Reinhard M., Erlandsen E.J., Randers E. Biological variation of cystatin C and creatinine. *Scand J Clin Lab Invest* 2009; 69(8): 831–836.

18. Mussap M., Dalla Vestra M., et al. Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. *Kidney Int* 2002; 61(4): 1453–1461.

19. Herget-Rosenthal S., Bokenkamp A., Hofmann W., et al. How to estimate GFR-serum creatinine, serum cystatin C or equations? *Clin Biochem* 2007; 40(3–4): 153–161.

20. Stevens L.A., Coresh J., Schmid C.H., et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis* 2008; 51(3): 395–406.

21. Oddoze C., Morange S., Portugal H., et al. Cystatin C is not more sensitive than creatinine for detecting early renal impairment in patients with diabetes. *Am J Kidney Dis* 2001; 38(2): 310–316.

22. Tidman M., Sjostrom P., Jones I., et al. A Comparison of GFR estimating formulae based upon s-cystatin C and s-creatinine and a combination of the two. *Nephrol Dial Transplant* 2008; 23(1): 154–160.

23. Rigalleau V., Raffaitin C., Gin H., et al. Accounting for body composition does not improve cystatin C estimation of GFR in diabetic subjects with CKD. *Am J Kidney Dis* 2007; 49(4): 560.

24. Perlemoine C., Beauvieux M.C., Rigalleau V., et al. Interest of cystatin C in screening diabetic patients for early impairment of renal function. *Metabolism* 2003; 52(10): 1258–1264.

25. Jerums G., Premaratne E., Panagiotopoulos S., et al. New and old markers of progression of diabetic nephropathy. *Diabetes Res Clin Pract* 2008; 82(Suppl 1): 30–37.

26. Shestakova M.V. Sakharnyy diabet i khronicheskaya bolezn' pochek: sovremennaya diagnostika i lechenie [Diabetes mellitus and chronic kidney disease: modern diagnostics and management]. *Vestnik RAMN — Herald of RAMS* 2012; 1: 45–49.

27. Shestakova M.V., Dedov I.I. *Sakharnyy diabet i khronicheskaya bolezn' pochek* [Diabetes mellitus and chronic kidney disease]. Moscow: OOO «Meditsinskoe informatsionnoe agentstvo»; 2009; 482 p.

28. Shestakova M.V. Sovremennoe ponyatie «Khronicheskaya bolezn' pochek»: metody diagnostiki, klinicheskoe znachenie [Modern concept of "Chronic kidney disease": diagnostic techniques, clinical relevance]. *Sakharnyy diabet — Diabetes Mellitus* 2008; 2: 4–7.

29. Maslova O.V., Suntsov Yu.I., Shestakova M.V. i soavt. Rasprostranennost' diabeticheskoy nefropatii i khronicheskoy bolezni pochek pri sakharnom diabete v Rossiyskoy Federatsii [The incidence of diabetic nephropathy and chronic kidney disease in diabetes mellitus in the Russian Federation]. *Klinicheskaya nefrologiya — Clinical Nephrology* 2010; 3: 45–51.

30. KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. National Kidney Foundation. *Am J Kidney Dis* 2007; 49(Suppl 2): L–180.