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ROLE OF CYSTATIN C IN THE DIAGNOSIS AND PROGNOSIS OF THE DEVELOPMENT OF CARDIORENAL SYNDROME IN COMORBID PATIENTS WITH ARTERIAL HYPERTENSION

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The purpose of our study was to determine of diagnostic and prognostic role of cystatin C in the development of cardiorenal syndrome in the case of arterial hypertension syndrome and maximum comorbid conditions. 111 patients and 20 persons of the control group were examined. In the current examination process, they were divided into 4 groups depending on the presence of comorbid pathology in them: patients with arterial hypertension – 1 group – 22 people; patients with arterial hypertension in combination with obesity – group 2 – 30 people; arterial hypertension in combination with type 2 diabetes – group 3 – 31 people; patients with hypertension, type 2 diabetes and obesity – group 4 – 28 people. The level of cystatin C in the examined cases with arterial hypertension and various comorbidities was significantly higher compared to individuals of the control group. Significant effect of the cardiotrophin-1 residue on cystatin C. An increased level of cystatin C is associated with an increase in blood pressure, the level of cardiotrophin-1, catestatin, which ensures its role in the early development of nervous and cardiovascular complications in patients with arterial hypertension with various hormonal comorbidities and confirms the significance of the activity of adipose tissue in the development of kidney dysfunction.

Key words: arterial hypertension, type 2 diabetes mellitus, obesity, cystatin C, cardiorenal syndrome.

І.П. Дунаєва

РОЛЬ ЦИСТАТИНУ С В ДІАГНОСТИЦІ ТА ПРОГНОЗУВАННІ РОЗВИТКУ КАРДІОРЕНАЛЬНОГО СИНДРОМУ У КОМОРБІДНИХ ПАЦІЄНТІВ З АРТЕРІАЛЬНОЮ ГІПЕРТЕНЗІЄЮ

Метою нашого дослідження було визначення діагностичної і прогностичної ролі цистатину С в розвитку кардіоренального синдрому у пацієнтів з артеріальною гіпертензією і різними коморбідними станами. Обстежено 111 хворих та 20 осіб контрольної групи. В процесі ретельного обстеження вони були розподілені на 4 групи в залежності від наявності в них коморбідної патології: хворі на артеріальну гіпертензію – 1 група – 22 особи; хворі на артеріальну гіпертензію в сполученні з ожирінням – 2 група – 30 осіб; артеріальна гіпертензія в сполученні з цукровим діабетом 2 типу – 3 група – 31 особа; пацієнти з артеріальною гіпертензією, цукровим діабетом 2 типу та ожирінням – 4 група – 28 осіб. Рівень цистатину С у обстежених пацієнтів з артеріальною гіпертензією та різною коморбідністю був значуще вищим у порівнянні з особами контрольної групи. Значущий вплив на цистатин С здійснює кардіотрофін-1. Підвищений рівень цистатину С асоційований з підвищенням артеріального тиску, рівнем кардіотрофіну-1, катестатину, що засвідчує його роль в ранньому розвитку ниркових і серцево-судинних ускладнень у хворих на артеріальну гіпертензію з різною коморбідністю та підтверджує значущість гормональної активності жирової тканини в розвитку дисфункції нирок.

Ключові слова: артеріальна гіпертензія, цукровий діабет 2 типу, ожиріння, цистатин С, кардіоренальний синдром.

The work is a fragment of the research project "To determine the features of immunocytokine imbalance in comorbid patients with arterial hypertension and type 2 diabetes mellitus and cardiovascular and renal complications", state registration No. 0123U101711.

Currently, early diagnosis of kidney dysfunction is based on biological markers that can be used for screening and diagnostic purposes to identify stages (condition biomarkers), risk assessment (antecedents), prognosis and outcome (predictive), and treatment efficacy.

These biomarkers include microalbuminuria (MA), proteins detected in urine (IgG, albumin, α1-microglobulin, α2-microglobulin, β2-microglobulin, α- and π-glutathione S-transferases, type VI collagen), compounds detected in blood (cystatin C (Cys C), prouroguanylin, proguanylin, lipocaine, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule, IL-18, antineutrophil cytoplasmic antibodies, asymmetric dimethylarginine, liver fatty acid binding protein, retinol-binding protein), which represent impaired glomerular and/or tubular renal functions [6, 8].

Cys C, a biomarker of renal glomerular function, holds a particular place in this series, as it also has unique properties as an independent predictor of cardiovascular disease [9, 12, 14]. Cys C falls into the family of papain-like cysteine protease inhibitors, and its biological role involves inhibiting cathepsins. In humans, Cys C is constantly secreted by all nuclear cells and is present in large quantities in all biological fluids. This is a non-glycosylated protein with a molecular weight of 13 kDa. Protein excretion is carried out by the kidneys. Cys C is freely excreted by glomerular filtration through the glomerular membrane and then undergoes complete tubular absorption through proximal tubular cells and catabolism. Serum Cys C concentration is inversely correlated with GFR, with Cys C being a marker of glomerular dysfunction even when creatinine is still within the normal range [1, 10].

The Cys C synthesis rate in the body is constant, and the excretion rate depends on renal pathology. It means the more severe the renal pathology, the worse Cys C is filtered in the kidneys and the higher its blood level. It is synthesized by all cells in the body that contain a nucleus. Researchers suggest considering gender, age, percentage of adipose tissue, smoking, and alcohol consumption when determining GFR with Cys C [7].

Back in 2004, Cys C was officially recognized by the FDA (U.S. Food and Drug Administration) as a marker for alternative GFR determination. There were recommendations for the calculation of GFR based on a single determination of Cys C in the blood serum.

Multiple studies have shown that Cys C is more than just a marker of kidney function, but also an independent risk factor for cardiovascular disease, heart failure, and mortality.

By now, several studies have proved a direct relationship between Cys C and functional and predictive indices of cardiovascular disease. There are studies that show that Cys C level, to a greater extent than creatinine, defines the risk of mortality in a variety of cardiovascular diseases, peripheral arterial disease, metabolic syndrome, and type 2 diabetes mellitus (T2DM), regardless of the deterioration of renal function.

The purpose of the study was to determine the diagnostic and prognostic role of cystatin C in the development of cardiorenal syndrome in the case of arterial hypertension syndrome and maximum comorbid conditions.

Materials and methods. The set of studies was carried out under the ethical and legal requirements of the Ukrainian Association for Bioethics and the GCP norms (1992), GLP (2002), the principles of the Declaration of Helsinki on Human Rights, the Council of Europe Convention on Human Rights and Biomedicine and approved by the Ethics and Bioethics Committee of Kharkiv National Medical University.

111 patients with AH (men/women – 50/61) and 20 control subjects were examined. All patients with AH at the age of 54.37±1.18 were treated at the clinic of the Government Institution "L.T. Malaya Therapy National Institute" of the National Academy of Medical Sciences of Ukraine. During a thorough examination and follow-up of patients, they were classified into 4 groups depending on the comorbidities they had: patients with AH – group 1 – 22 persons; patients with AH in combination with obesity – group 2 – 30 persons; AH in combination with type 2 diabetes – group 3 – 31 persons; patients with AH, type 2 diabetes and obesity – group 4 – 28 persons.

Body weight and height were measured in all patients; BMI = body weight/height^2 (m^2) was calculated; systolic and diastolic blood pressure was measured.

Cys C, cardiotrophin-1, catestatin, β2-microglobulin, leptin, neutrophil gelatinase-associated lipocalin (NGAL), 25-OH total vitamin D (Vitamin D3), and serum insulin levels were measured by enzyme-linked immunosorbent assay on a Labline-90 analyzer (Austria) with commercial test systems manufactured by Fine Test (ELISA, China), BT LAB (ELISA, China), DBC (ELISA, China), Elabscience (ELISA, Canada), Monobind Inc. (ELISA, USA), according to the instructions included in the kits.

Biochemical studies (creatinine, urea, serum lipid spectrum, and glycated hemoglobin level) were performed on a Labline-90 analyzer (Austria). Serum urea levels were measured by the kinetic enzymatic method with urease/glutamate dehydrogenase using Liquick Cor-UREA 30 kits (Cormay, Poland)

according to the manufacturer's instructions. The serum creatinine level was measured by the modification of the Jaffe method without deproteinization using Liquick Cor-CREATININ 30 reagent kits (Poland) according to the manufacturer's instructions. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG) were determined by the enzymatic method using reagent kits Cholesterol liquicolor, HDL–Cholesterol, and Triglycerides liquicolor (Human, Germany) according to the manufacturer's instructions. The content of very low-density lipoprotein (VLDL) was calculated using the TG/2.22 formula; the content of low-density lipoprotein (LDL) was calculated using the W. T. Friedewald formula, 2004:

LDL=TC-(HDL+TG/2.22), mmol/l.

Cys C content in the blood serum was determined by enzyme-linked immunosorbent assay using a Labline-90 analyzer (Austria) and a commercial test system manufactured by Elabscience (ELISA, China) according to the instructions included in the kit.

The following were the exclusion criteria for the study: Type 1 diabetes mellitus, congenital heart and urinary tract defects, artificial pacemakers, artificial heart valves, stage II B and III heart failure, acute heart attack, infectious and severe inflammatory processes, and hematological diseases.

Statistical data analysis was performed using Statistica, 12 (Stat Soft Inc, USA) and Microsoft Office Excel 2013. The data are presented as mean (M) and standard deviation (δ). Differences between groups of mean values were evaluated using the Student's t-test. An error of less than 5 % ($p < 0.05$) was considered reliable. Stepwise regression analysis was used to determine the degree of influence of the studied indicators on the CTF-1 level with the evaluation of its effectiveness using one-way analysis of variance (ANOVA).

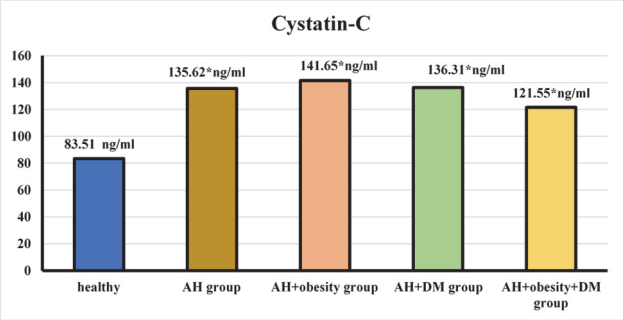

Fig. 1. Cystatin C values in the examined groups of patients.

Results of the study and their discussion. According to the study, the Cys C level in the examined patients with AH and various comorbidities was significantly higher compared with the control group, with $p < 0.0001$ (Fig. 1).

According to the univariate and multivariate regression analysis, the factors affecting the Cys C level were identified (Table 1).

Table 1

Factors affecting the Cys C level

Indices	Dependent component: Cys C **(Y)**							
	Univariate linear regression analysis (χ^2=19.41; p = 0.022)				Multivariate linear regression analysis (χ^2=29.72; p = 0.0018)			
	β-coefficient	DOR	95% CI	P	β-coefficient	DOR	95% CI	P
Cardiotrophin-1, pg/ml	-0.004	0.996	0.993- 0.999	0.0169	-0.004	0.996	0.993-0.999	0.015
Catestatin, ng/ml	0.514	1.672	0.889-3.144	0.1107	0.556	1.744	0.938-3.242	0.079
Leptin, ng/ml	0.039	1.040	0.995- 1.088	0.0856	0.037	1.038	0.995-1.083	0.084
Glycated hemoglobin, %	0.521	1.684	0.818- 3.466	0.1570	0.588	1.801	0.901-3.597	0.096
β2-microglobulin, μg/ml	-0.385	0.680	0.395- 1.171	0.1646				
Vitamin D3, ng/ml	0.022	1.023	0.979- 1.068	0.3140				
Obesity	0.754	2.125	0.647- 6.978	0.2140	0.904	2.469	0.777-7.850	0.126
Creatinine, μmol/l	0.013	1.013	0.980- 1.046	0.4493				
TG, mmol/l	0.337	1.401	0.703- 2.793	0.3382				

We found that cardiotrophin-1, a versatile biomarker for the development and progression of cardiovascular disorders in patients with AH and various comorbidities, has a significant impact on this index (p=0.015).

The first place among the properties of cardiotrophin-1 in the body is its role in the regulation of heart remodeling in patients with hypertension, heart failure, coronary heart disease in combination with T2DM and hypertension. The resulting relationship between cardiotrophin-1 and Cys C can testify to the latter's contribution to the development of cardiovascular complications in patients with AH, T2DM, and obesity.

As part of the study, we performed a regression analysis to determine the relationship between Cys C and the investigated parameters separately for the groups of patients.

Thus, there was a significant correlation of Cys C with insulin (p=0.029) and β2-microglobulin (p=0.040) in the group of patients with AH without concomitant diseases. A significant correlation of Cys C with diastolic blood pressure (p=0.027) and BMI (p=0.024) was demonstrated in the group of patients with AH and concomitant obesity. A significant correlation of cystatin C with β2-microglobulin (p=0.013), urea (p=0.017), and systolic blood pressure (p=0.042) was demonstrated in the group of patients with AH and T2DM. A significant correlation of Cys C with cardiotrophin-1 (p=0.0026), catestatin (p=0.0046), and insulin (p=0.027) was demonstrated in patients with more severe comorbidity of AH+T2DM+obesity (Table 2).

Table 2

Impact of various factors on the Cys C level in the examined patients with AH (group 1), AH+ obesity (group 2), AH+T2DM (group 3), AH+T2DM+obesity (4) (regression analysis)

Predictor	AH		AH + obesity		AH + T2DM		AH + T2DM + obesity		p level
	I		II		III		IV		
	B	Wald test	B	Wald test	B	Wald test	B	Wald test	$p_{I.\ II}$ <0.05
									$p_{III.\ IV}$ <0.01
DBP, mmHg	0.37	12.444	1.879	10.228	–2.136	12.652	–8.764	14.256	$p_{I.\ II}$ <0.03
									$p_{III.\ IV}$ <0.002
Insulin, μU/ml	–2.533	10.673	–2.058	9.772	–1.949	12.363	–8.654	12.689	$p_{I.\ II}$ <0.001
									$p_{III.\ IV}$ <0.005
β2-micro-globulin, μg/ml	–18.323	13.244	–12.634	11.518	–3.644	10.125	0.722	8.124	$p_{I.\ II}$ <0.01
									$p_{III.\ IV}$ <0.001
Urea, mmol/l	0.487	12.654	0.86	8.121	0.88	13.711	0.894	13.905	$p_{I.\ II}$ <0.025
									$p_{III.\ IV}$ <0.05
SBP, mmHg	0.37	10.541	2.121	6.478	1.322	11.544	–4.697	12.856	$p_{I.\ II}$ <0.05
									$p_{III.\ IV}$ <0.025
Leptin, ng/ml	1.098	9.211	0.711	11.956	1.058	10.681	1.078	10.689	$p_{I.\ II}$ <0.05
									$p_{III.\ IV}$ <0.05
NGAL, ng/ml	0.531	11.781	0.954	10.871	–3.263	12.601	2.424	9.865	$p_{I.\ II}$ <0.05
									$p_{III.\ IV}$ <0.05
TG, mmol/l	0.22	4.857	0.65	7.654	4.888	13.261	–0.815	11.862	$p_{I.\ II}$ <0.05
									$p_{III.\ IV}$ <0.025
Glycated hemoglobin, %	0.0778	8.781	0.259	12.734	8.953	14.121	0.043	8.781	$p_{I.\ II}$ <0.05
									$p_{III.\ IV}$ <0.05

The data we obtained reveal the relevance of Cys C for the development of both renal episodes and metabolic changes, as well as its contribution to cardiovascular disease.

This study shows that Cys C is associated with cardiotrophin-1, catestatin, blood pressure, insulin, and β2-microglobulin. Cys C is an inhibitor of cysteine proteases. It is constantly secreted by all nuclear cells, including adipose tissue cells, and is fully filtered and not secreted by the proximal tubules [13]. On the one hand, increased Cys C in adipose tissue has a protective effect by blocking cathepsin proteases. On the other hand, it reduces the proliferation of adipose tissue in a way similar to atherosclerosis by inhibiting cysteine proteases and prevents the development of atherosclerotic damage in the vascular wall [3, 5, 11].

In a study on the relationship between Cys C and the frequency of metabolic syndrome among a large cohort of patients (more than 2,000), the previously proven relationship between high levels of Cys C and an increased risk of future development of metabolic syndrome was reproduced, but the genetic increase in the level of Cys C in the plasma of the examined subjects was not was associated with the frequency of metabolic disorders and T2DM [11].

An experimental study reported that circulating levels of Cys C were positively correlated with obesity in humans, as mice fed a special high-fat diet (HFD) thereby improved adipose tissue and hepatic insulin sensitivity [3]. The authors concluded that Cys C is upregulated in obesity, and thus counteracts inflammation of peripheral insulin-sensitive tissues and thus obesity-related impairment of glucose metabolism. In patients with T2DM and nephropathy, a significant deterioration in the level of Cys C in blood serum ($p < 0.01$) was detected simultaneously with significant disturbances in lipid metabolism [4]. Cys C level, systolic blood pressure, fasting blood glucose, total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, and duration of diabetes were found to be significantly associated with diabetic nephropathy ($P < 0.05$). It was concluded that fasting blood glucose level and lipid profile are significantly related to Cys C level.

Thus, the presented results of our research and the analysis of the works of other scientists confirm the importance of Cys C. This biomarker is especially important in people with the so-called cardiorenal syndrome. This syndrome got its name in connection with the combination of diseases of the heart and kidneys associated with the commonality of their pathogenesis, the presence of common risk factors and mutual aggravation. Cys C can be considered a marker of the development of cardiorenal syndrome in comorbid patients [2, 5].

Among the major risk factors for the development of cardiorenal syndrome in comorbid patients with AH are hypertension, obesity, and carbohydrate metabolism disorders, so Cys C can be considered a marker for the development of cardiorenal syndrome in such patients.

Conclusions

1. Serum cystatin C level is a reliable marker of glomerular filtration rate with high diagnostic accuracy, which improves risk stratification for the development of chronic kidney disease in comorbid patients with AH.

2. Elevated cystatin C level may be associated with increased blood pressure, cardiotrophin-1 level, and serum catestatin value, which indicates its importance for the early development of cardiovascular complications in patients with AH and various comorbidities and proves the significance of adipose tissue hormonal activity for the development of glomerular and tubular dysfunctions.

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