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The Functional State of the Kidneys and Endothelial Dysfunction in Patients with Arterial Hypertension

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ABSTRACT

Endothelial dysfunction (ED) is the leading pathogenetic link connecting the development of atherosclerosis, diabetes mellitus (DM) type 2, arterial hypertension (AH) and chronic kidney disease (CKD). In this regard, the integrated assessment of markers of endothelial dysfunction in patients at the early stages of hypertension based on kidney function seems important. The survey included 67 patients with hypertension (mean age 48.2±2.4 years) and 30 matched for sex and age healthy volunteers. The levels of markers of endothelial dysfunction of blood VEGF, endothelin-1 and renal damage - MAU, VEGF in the urine - were significantly higher in the group with AH. Correlation analysis showed the existence of a high degree of direct connection between the level of VEGF in the blood and the arterial pressure value, the degree of obesity, cholesterol, creatinine, MAU and negative correlation with VEGF and glomerular filtration rate (GFR). We also found a positive correlation of endothelin-1 and VEGF in the blood. Similarly, there was a positive relationship between the levels of VEGF in the urine and the systolic AH, MAU, uric acid, and the negative one - with GFR. The value of the MAU is directly dependent on the level of diastolic blood pressure and creatinine, and it was inversely proportional to GFR value. Thus, in patients with hypertension endothelin-1 and VEGF in the blood are markers of endothelial dysfunction responsible for the adverse cardiovascular prognosis, and the level of MAU and VEGF in urine correlating with the degree of decrease in kidney function and high blood pressure are markers of adverse cardio-renal relationship.

Key words. arterial hypertension, endothelial dysfunction, kidney diosease.

INTRODUCTION

Today, the world's leading pathogenetic link connecting the development of atherosclerosis, diabetes mellitus (DM) type 2, arterial hypertension (AH), chronic kidney disease (CKD) is endothelial dysfunction (ED)[1]. Endothelial dysfunction is characterized by shear in the endothelium in the direction of decreasing vasodilation as well as by proinflammatory and prothrombotic state[2]. Numerous studies have shown a close relationship between insulin-resistance (IR) and endothelial dysfunction, manifesting by decreased activity of nitric oxide (NO), the decrease in the formation of prostacyclin and increased production of vasoconstrictor substances[3]. Integral marker for cardiorenal interaction is microalbuminuria (MAU), the presence of which indicates impaired endothelial function[4].

Currently, among the mechanisms of progression of glomerular lesions of the kidney belongs to ED. It has been proven that the renal artery are characterized by the highest, compared

with other organs, sensitivity to endothelin-1, which is a major vasoconstrictor peptide. In addition, endothelin-1 is considered as a causal factor in the development of renal fibrosis[5]. Vascular endothelial growth factor (VEGF) participates in the development of atherosclerosis, hypertension, and type 2 diabetes[6,7]. In some works it was shown that VEGF is an early marker of renal damage in patients with hypertension[6,8].

Presented data indicate a prominent increase of VEGF level in chronic pyelonephritis, increasing with disease progression earlier than the fall in GFR, that can be considered as a prognostic indicator[9]. Thus, detection of early signs of ED depending on the functional state of kidneys in patients with arterial hypertension is very important.

The aim of the study was to evaluate the existence of ED in hypertensive patients depending on the functional state of the kidneys.

MATERIALS AND METHODS

The survey included 67 patients with metabolic syndrome (mean age 48.2±2.4 years) with arterial hypertension of 1-2 degrees, stage I-II, without organic damage to the kidneys in anamnesis and pathological changes in general urine analysis, creatinine, without ultrasound signs of anatomical and structural changes of the kidneys. All patients had not previously received antihypertensive therapy and statins. The study did not include patients with secondary forms of hypertension, patients with hypertension III stage, 3rd degree, diabetes mellitus (DM), inflammatory diseases of the myocardium, systemic connective tissue diseases, and cancer. The comparison group consisted of 30 healthy volunteers (mean age of 39.4±5.3 years).

All participants underwent standard clinical and laboratory examination. The levels of lipids, glucose, creatinine, uric acid, transaminases and bilirubin were determined by standard biochemical methods. BMI was calculated by the Quetelet formula: weight (kg) / height (m^2).. GFR was calculated using the formula CKD-EPI (ml/min/ 1.73 m^2). The degree of decline in GFR was assessed in accordance with the recommendations[9].

The levels of VEGF in the blood and urine, endothelin-1 in blood, MAU were determined by enzyme immunoassay (ELISA) using a photometer "Stat Fax" ("Awareness Technology Inc.", USA) and the corresponding reagents ZAO "Vector-best" (Novosibirsk), "Biomedica" Austria, ELISA micro-albumin produced by Orgentec, Germany.

In statistical data processing we used the program "Statistica 7.0 Rus". Evaluating data with the normal distribution used (mean, standard error of the mean), *t* student test. Data with abnormal distribution are presented as median and interquartile range [25;75]. To compare unrelated samples on quantitative indicators we used nonparametric test Mann-Whitney test. Differences between samples were considered significant at the value $p<0.05$. Communication traits were assessed using regression analysis with determination of the coefficient of the Spearman rank correlation; the relationship between values was assessed as strong for R >|0,7|, the average power at R from |0,3| to |0,7|, and weak for R< |0,3|.

RESULTS

Main group and the comparison group did not differ by age and sex. The average duration of hypertension was 4.1±2.5 years; family history of cardiovascular disease (CVD) was identified in all patients of the main group. Dyslipidemia with hypertriglyceridemia was present in 90%, hyperglycemia (glycemia > 6.1 mmol/l) was found in 68.5 % of patients. Patients of the main group compared with the comparison group revealed elevated levels of cholesterol, triglycerides, low density lipoproteins, glucose, and uric acid (Table 1).

There were correlations between the size of waist circumference (WC) and the level of diastolic blood pressure (r=0.43; p=0.03), uric acid (r=0.5; p=0.04), triglycerides (r=0.36; p=0.03) and very low density lipoproteins (VLDL) (r=0.5; p=0.04). Creatinine was associated with total cholesterol (r=0.42; p=0.045), VLDL (r=0.5; p=0.04) and uric acid (r=0.48; p=0.03).

Optimal GFR (>90 ml/min/1.73m^2) was detected in 40.3% (n=27) of patients, again – 40.3% (n=27) showed a slight decline (>60 to <90 ml/min/1.73m^2) and 4.4% (n=3) moderate decline (>45 to<60 ml/min/1.73m^2), 15 % (n=10) revealed increased GFR. A significant increase in GFR and the decrease was marked in patients with obesity of the 3rd degree. A negative relationship was observed in estimated GFR with indices of systolic blood pressure (r=-0.47; p=0.03) and diastolic blood pressure (r=-0.37; p=0.04), and glucose concentration (r=-0,28 ; p=0.03). Indicators of endothelial dysfunction: VEGF in the blood, endothelin-1, MAU, VEGF in the urine were significantly higher in the AH group (Table 2).

We also revealed a direct high degree correlation between the level of VEGF in the blood and total cholesterol (r=0.74; p=0.02), creatinine (r=0.67; p=0.001), BMI (r=0.4; p=0.04), the level of diastolic blood pressure (r=0.38; p=0.04), systolic blood pressure (r=0.6; p=0.02), MAU (r=0.73 ; p=0.02) and negative correlation of VEGF and EGFR (r=-0.7; p=0.02). Endothelin-1 had a reliable correlation with the level of VEGF in the blood (r=0.67; p=0.01).

Pathological MAU level (>30 mg/ml) was found in 31.3% (n=21) patients, 44.7% had elevated levels of MAU (10-29 mg/ml) (n=30). We found a positive correlation of values of MAU with the level of diastolic blood pressure (r=0.7; p=0.02),

Table 1: Comparison of metabolic parameters in groups (Ì ± SD)

Parameters	Obesity group(n=67)	Healthy persons(n=30)	P
Age (years)	48.2±2.4	39.4±5.3	0.7
WC (cm)	113.2±11	80.7±11	0.0001
BMI (kg/m^2)	38.0±9	26,1±1,3	0.00001
SBP (mm Hg)	147.9±7.1	128,3±7,2	0.0001
DBP (mm Hg)	98.8±5.0	78,5±5,3	0.0001
Glucose (mmol/L)	5.8±0.8	4.21±0.5	0.01
TC (mmol/L)	5.9±0.9	4±0.7	0.04
VLDL (mmol/L)	3.62±0.7	2,43±0,4	0,03
HDL (mmol/L)	1.31±0.3	1.4±0.1	0.3
TG (mmol/L)	2.33±1.1	0.57±0,11	0.001
Creatinine (mcmol/L)	78.6±12.5	66.0±10.1	0.01
Uric acid (mmol/L)	391.4±45.4	241.5±86.1	0.009
GFR ml/min/1.73 m^2.	88.5±5.8	112.7±7.6	0.001

Abbreviations: AG - arterial hypertension; WC - waist circumference; BMI – body mass index; SBP – systolic blood pressure; DBP- diastolic blood pressure; TC- total cholesterol; VLDL – very low density lipoproteins; HDL- high density lipoproteins; TG- triglycerides; GFR - glomerular filtration rate.

Table 2: Concentration of biomarkers of endothelial dysfunction in groups

Parameter	Median value (25; 75% the percentile); [Min- and Max- parameter value]		
	AH (n = 67)	Control (n = 30)	P
Microalbumin, mg/ml	25.8 (11.7; 34.0); [8.6; 56.5]	9.4 (5.4;13.2); [1.4; 20.0]	0.001
VEGF, pg/ml (urine)	79.0 (28; 96,2); [5,0; 342]	15.8 (9.1; 21.7); [2.1; 46.4]	0.0001
VEGF, pg/ml (blood)	195 (96.2; 288); [5-961]	75 (0-96); [0129]	0.01
Endothelin-1, fmol/ml	2.7 (0.3-2.8) [0-6.1]	0.5 (0.1-0.5) [0-2.1]	0.01

creatinine level (r=0.42; p=0.03), total cholesterol (r=0.41; P=0.04), VEGF (r=0.36; P=0.03), and negative one with a value of GFR (r=-0.5; p=0.03). We also detected a direct relationship between the level of VEGF in the urine and systolic blood pressure (r=0.49; p=0.03) and MAU (r=0,47; p=0.03), uric acid (r=0,49; p=0.04), and a negative one with EGFR (r=-0.46; p=0.03).

The study of markers of endothelial dysfunction depending on the functional state of the kidneys revealed an increase in MAU and the level of VEGF in the urine while kidney decline. However, significant changes of VEGF and endothelin-1 in the blood were not established (Table 3).

Table 3: Indicators of endothelial dysfunction depending on the functional state of the kidneys

Parameter	**Median value (25; 75% the percentile); [Min- and Max- parameter value]**			
	GFR>90 ml/min/1.73 m². **(n = 37)** **group**	**GFR<90>60 ml/min/1.73 m².** **(n = 27)** **2 group**	**GFR <60 >45 ml/min/1.73 m².** **(n = 3)** **3 group**	**p**
Microalbumin, mg/ml	21 (11.0; 34.0); [5.0; 45.0]	29 (21.0; 37.0); [10.0; 56]	30 (24.0; 56.6) [24; 56.6]	p1-2 0.04 p1-3 0.04 p2-3 n.d.
VEGF, pg/ml (urine)	48,0 (14; 96,2); [5.0; 235]	85 (35; 109); [5,0; 342]	80 (21; 193); [21; 193]	p1-2 0.03 p1-3 0.03 p2-3 n.d.
VEGF, pg/ml (blood)	129 (76-227); [0-882]	160 (82-288); [0-800]	158 (100-224); [100-224]	n.d.
Endothelin-1, fmol/ml	2,5 (0,3-2,8) [0-5.1]	2,2 (0.3-3.6) [0-6.1]	2,2 (0,3-3,6) [0,3-3,6]	n.d.

Note: P1-2 differences between the group with GFR>90 ml/min/1.73m² and the group with GFR<90>60 ml/min/1.73 m²; P1-3 differences between the group with GFR>90 ml/min/1.73m² and the group with GFR<60 ml/min/1,73m²; P2-3 differences between the group with GFR<90>60 ml/min/1. 73m² and the group with GFR<

DISCUSSION

At present, indisputable is the concept of total cardiovascular risk, which considers ED as a manifestation of systemic atherosclerosis. Increased blood pressure leads to disruption of the architectonics of endothelial cells, increasing their permeability to albumin, increased secretion of the vasoconstrictor endothelin-1, remodeling of the vascular walls [2]. Endothelial dysfunction primarily affects the kidney, resulting in disorders of renal hemodynamics, activation of intravascular coagulation, and renal fibrosis. These events for a long time may be asymptomatic, which contributes to late diagnosis of kidney dysfunction.

Our study has shown that 31% of patients with hypertension that do not have data for renal damage, have MAU, and of 44.7% - decrease in GFR. To date, GFR is not only seen in the context of renal injury, but also as a risk factor for adverse outcomes in persons with cardiovascular disease (CVD) and without them. The ALLHAT study showed that in hypertensive patients with one or more CV risk factors, optimal GFR is determined only 15% of patients while 73.9% of patients have decreased GFR (the average age of respondents was 66 years). In some works it was shown that with decrease of GFR by 5 ml/min/1.73 m² the risk of cardiovascular mortality is increased by 26% and reduction in GFR from 90 to 60 ml/min/1.73 m² increases it in 4 times. Moreover, the relationship of decreasing GFR with CVD outcomes was independent of the presence of hypertension, DM, prior CVD, lipid profile, markers of endothelial dysfunction[2]. In our study, a smaller percentage of

patients with decreased GFR - 44.73% - can be explained by their young group (mean age 48 years), and by little experience of AH - 2-4 years.

It was also shown that young patients with little experience of AH had a significant increase in VEGF level[6, 8]. In our study there was a correlation of VEGF level of SBP and DBP, which confirms the presence of endothelial dysfunction in patients with low experience and low degree of AH. Thus, the elevated levels of VEGF in patients with hypertension is an important marker of endothelial dysfunction responsible for progression of the disease.

The obtained results seem logical, since it is known that VEGF regulates the tone of the vascular wall, supporting spastic processes and an increase in the concentration causes abnormal vasospasm. It is the most important factor in the regulation of glomerular barrier function in health, and while disease its products contribute to the development of sclerotic process and the progression of chronic kidney disease[10, 11].

Recent studies have shown that a system endothelins makes a significant pathogenic contribution to the formation of glomerulosclerosis, tubulointerstitial fibrosis, and to the severity of hypertension. It has been proven that angiotensin II stimulates the excretion of endothelin-1 [4, 5]. In our study, in the group of AH we identified an increased level of endothelin-1; in addition, we detected a reliable strong correlation with the VEGF level in the blood. These data suggest that patients with a mild form and a little experience of AH, there are signs of endothelial dysfunction. The lack of significant correlations between the concentration of endothelin-1 and blood pressure, indices of metabolism, the lack of changes in concentrations while lowering of kidney function can explain the low seniority and mild hypertension and no diagnosis of chronic kidney disease (CKD). Significant correlations of endothelin-1 and reduced renal function were obtained in form of CKD and while hypertension with a high degree of increase in blood pressure[1, 5].

These modern data interprets MAU as a reliable marker of renal dysfunction and a manifestation of general lesions of the microvascular channels and total cardiovascular risk. On average, at AH, according to the literature data, MAU detected in 30-40% of patients, and in some cases - in 72% of patients, which is probably due to the duration and severity of the disease[2, 4]. Data our study (the frequency of MAU in 31.6% of patients) corresponds to the literature data. It was found that the decrease in GFR was accompanied by an increase in MAU, which confirms the presence of renal damage.

A number of studies have shown that the link between MAU and cardiovascular risk may be excess production of VEGF, which increases vascular permeability, contributing to hemorrhagic and atherosclerotic processes, and also increases the glomerular filter permeability for albumin[7, 8, 9]. It was shown that in patients with mild form of hypertension a marker of early renal damage is also VEGF, and there was a direct correlation between the size of the MAU and the level of VEGF in the urine[6, 10]. The obtained data reflect both the presence of endothelial dysfunction, and activation of mechanisms of fibrogenesis, which are parts of processes of remodeling microvascular channels of the kidney in hypertensive nephropathy[8, 10].

In our study, the VEGF level in the urine was significantly higher in the group of AH; we also revealed the relationship between levels of MAU and VEGF. Moreover, it was found that in process of decrease of kidney function in the urine increases the concentration of MAU and VEGF, which confirms the presence of endothelial dysfunction, activation of mechanisms of fibrogenesis of the glomerular apparatus of the kidney and associated decline in renal function. Similar results were obtained by other researchers[5, 6, 10, 11].

CONCLUSIONS

1. In patients with hypertension endothelin-1 and VEGF in blood were the markers of endothelial dysfunction, which is an unfavorable factor for cardiovascular prognosis.
2. In patients with hypertension, the levels of MAU and VEGF in the urine correlate with the degree of reduction of renal function and

the degree of increase in blood pressure, which is unfavorable prognosis in relation to cardio-renal relationship.

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