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EARLY STAGE OF RENAL PATHOLOGY IN PATIENTS WITH ESSENTIAL ARTERIAL HYPERTENSION: DIAGNOSTIC AND MONITORING

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Basing on the survey of 259 patients with essential arterial hypertension (EAH) early clinical diagnostic markers of involvement in the pathological process of the kidneys have been defined. The survey, which included assessment of microalbuminuria, glomerular filtration rate, investigation of indicators of renal hemodynamics by Doppler method and study of urinary excretion of molecular mediators that reflect pathophysiological processes in the kidney in patients with EAH, helps to set the dynamic characteristics of early hypertensive nephropathy and to define complex of clinical diagnostic markers of the stages of evolution of hypertensive vascular nephropathy. The earliest signs of involvement in the pathological process of the kidneys in patients with EAH are increasing of urinary biomarkers of endothelial dysfunction closely associated with its remodeling of the extracellular matrix of the kidney and microalbuminuria. We can assess the progression of hypertensive kidney disease with the formation of maladaptive remodeling of intrarenal vascular and growth of ischemia of renal kidney by increase of resistance index of interlobar renal arteries, the downward trend in GFR (less than 90 ml/min/1.73 m^2), along with increase of urinary excretion of markers of fibrogenesis in the kidney, particularly of collagen of IV type in EAH patients with persistent microalbuminuria. It was noted that the decrease of GFR less than 60 ml/min/1.73m2 is recognized marker of subclinical dysfunction of the kidney in patients with EAH, it is first evidence of the development of chronic kidney disease in these patients that allows to relate this category of patients to group of high risk of progression of hypertensive kidney disease and requires active nephroprotective strategy.

Keywords: essential hypertension, kidney disease, resistance index, urinary biomarkers

На основании обследования 259 больных эссенциальной артериальной гипертензией (ЭАГ) определены ранние клинико-диагностические маркеры вовлечения в патологический процесс почек. По результатам обследования, включавшего оценку микроальбуминурии, расчетной скорости клубочковой фильтрации, определение показателей внутрипочечной гемодинамики допплерометрическим и изучение мочевой экскреции молекулярных медиаторов, отражающих патофизиологические процессы в почке у больных ЭАГ, установлен динамический характер ранней стадии гипертонической нефропатии и определен комплекс клинико-диагностических маркеров этапов эволюции гипертонической сосудистой нефропатии. К наиболее ранним признакам вовлечения в патологический процесс почек у больных ЭАГ относятся увеличение мочевых биомаркеров эндотелиальной дисфункции, тесно сопряженных с ней процессов ремоделирования экстрацеллюлярного матрикса почки, и микроальбуминурия. О прогрессировании гипертонической нефропатии с формированием дезадаптивного ремоделирования внутрипочечных сосудов и усилении ишемии почечной ткани можно судить по увеличению индекса резистентности междолевых почечных артерий, тенденции к снижению СКФ менее 90 мл/мин/1,73м^2 наряду и нарастанию экскреции с мочой маркеров фиброгенеза в почке, прежде всего коллагена IV типа, у пациентов ЭАГ с персистирующей микроальбуминурией. Отмечено, что снижение СКФ менее 60 мл/мин/1,73м^2 — общепризнанный маркер субклинического поражении почки у больных ЭАГ, скорее свидетельствует о развитии у этих пациентов хронической болезни почек, что позволяет отнести эту категорию пациентов в группу риска прогрессирования гипертонической нефропатии и требует проведения у них активной нефропротективной стратегии.

Ключевые слова: эссенциальная артериальная гипертензия, поражение почек, индекс резистентности, мочевые биомаркеры

Introduction

According to the summary statistics arterial hypertension (AH) is one of the leading causes of terminal renal failure in patients on dialysis [1-3], which makes the problem of studying of the renal pathology associated with hypertension essential. According to modern concepts central role of the development of hypertensive nephropathy (HNP) is non-immune renal vascular lesion [4,5]. High hypertension leads to the breakdown of autoregulation of intrarenal blood flow through hemodynamic factors, activates / disables the renal endothelial cells causing their dysfunction. Locally renal endothelial dysfunction in EAH and closely associated with it processes of remodeling of the extracellular matrix are responsible for structural and functional reconstruction of vascular bed. Initially eutrophic and then hypertrophic remodeling of renal arteries and arterioles potentiate the ischemia of renal tissue. Long-term persistence of systemic and intrarenal hypertension leads to the maladaptive remodeling of renal microvascular bed with emptying of intraglomerular and peritubular capillaries, which constitutes the pathophysiological basis of hypertensive vascular nephropathy [6,7]. Currently clinical and experimental studies confirmed informative value of study of the molecular mediators of endothelial dysfunction, endothelium-dependent part of hemostasis and profibrinogenic growth factors in the urine of patients with chronic glomerulonephritis for evaluating process activity and prognosis of the disease, in diabetes — for the early diagnosis of diabetic nephropathy [8-14]. In light of this concept refinement of the hemodynamic and molecular-cellular pathways of early GNP including a study of urinary excretion of molecular mediators of intercellular and cell-interactions of matrix in the kidney in patients with EAH, is primarily important to identify more effective measures of the pathogenetic effects of the disease as a whole.

The aim of the study was to characterize the early stage of renal disease in essential arterial hypertension (EAH), to determine methods for its diagnosis and monitoring.

Material and methods of research

259 previously untreated patients with EAH: 186 men and 73 women, average age — 46 (30, 53) years (here and hereafter, the median and interquartile range were 25 and 75 percentiles) have been examined. The group included 47 young people from 17 to 28 years, mean age — 20.6 (19, 22) with well-documented onset of hypertension and a little experience. The control group consisted of 57 healthy individuals matched by age and sex with patients from the basic group: 38 men and 19 women from 20 to 63 years, average age — 42 (31, 49).

Verification of the diagnosis of EAH was carried out in accordance with Russian guidelines for diagnosis and treatment of hypertension of Society of Cardiology of Russian Federation, 2008 (third revision) [15]. To characterize the changes in the kidneys we examined in all patients daily albuminuria by enzyme immunoassay (ELISA), the glomerular filtration rate — GFR by Cockcroft-Goulta formula adjusted to standard body surface area (ml/min/1.73m2). Assessment of renal hemodynamics was carried out basing on the results of ultrasound Doppler of renal interlobar arteries which was performed on the unit SSD-5500 (Aloka, Japan). As signs of increased resistance of intrarenal blood flow in accordance with the results of earlier studies we assumed an increase in the resistance index (RI > 0.65) [16]. In the urine of patients with EAH enzyme immunoassay (ELISA) determined the concentration of molecular mediators: inhibitor of plasminogen activator (PAI-I) — a marker of endothelial dysfunction and state of proteolysis / fibrinolysis in the kidney (Technozim PAI-I Antigen, Austria (PAI-I), n = 53), transforming growth factor-β1 (TGF-β1) — a key profibrogenic mediator («DRG MTPL» EIA-1864, Germany, n = 70), vascular endothelial growth factor (VEGF) — the regulator of endothelial proliferation and vascular permeability («BioSource International, Immunoassay Kit », USA, Belgium, n = 71) and collagen of IV type in 36 patients — a structural component of endothelial and epithelial basement membranes and extracellular matrix («Biotrin International, LTD, Daiichi Fine Chemical Co, LTD», Ireland, Japan). Results of the study was assessed by parametric and nonparametric methods using the software package STATISTICA (version 7.0). Quantitative data are presented as median and interquartile (25-75th percentile) range. Differences were considered significant at $p < 0.05$.

Results and discussion

1. Characteristics of the kidneys in patients with EAH

All patients with EAH were divided into 2 groups depending on the presence or absence of microalbuminuria (MAU) as an early marker of kidney damage. Among 259 patients with EAH MAU was detected in 186 patients (72%) of its level averaged 51.73 (38, 67) mg / day, in the rest 73 patients albuminuria did not reach the degree of MAU averaging 22.4 (18; 26) mg / day.

GFR was calculated in 212 cases of all patients; among 59 patients without MAU total filtration function of the kidneys was higher than that among 153 patients with MAU, respectively 108 (94; 125) and 96.2 (79, 116) ml/min/1.73 m ² (U = 6,382, p = 0,001). To clarify the nature of the change in GFR depending on MAU we compared the frequency of detection of normofiltration (GFR from 90 to 130 ml/min/1.73m2), hyperfiltration (GFR> 130 ml/min/1.73m2) and hypofiltration (GFR < 90 ml / min / 1.73 m ²) in groups of EAH patients with and without MAU. The frequency of normal and high GFR in these groups were not significantly different, whereas the decrease in GFR — hypofiltration was detected significantly more often in patients with MAU (Table 1). It is noteworthy that the decrease in GFR < 60ml/min/1.73m2 that corresponds to the criteria of renal damage in hypertension [15] were revealed only 3% (7 out of 212). It was established that the decrease of GFR in patients with EAH apparently for long time was not accompanied with increased serum creatinine, thus in patients with hypofiltration in our research it averaged 99 (87.2-111.5) umol / L).

Table 1

Frequency of normal, increased and decreased GFR in groups of patients with EAH with and without MAU

GFT	MAU (-) (n = 59)	MAU (+) (n = 153)	Reliability
Hyperfiltration, n = 49	16 (27%)	33 (21,5%)	$\chi^2 = 0,72$; p = 0,39
Normofiltration, n = 91	30 (51%)	61 (40%)	$\chi^2 = 0,74$; p = 0,15
Hypofiltration, n = 72	13 (22%)	59 (38,5%)	$\chi^2 = 5,19$; p = 0,023

Calculating the probability (chance) of impairment of renal function in patients with EAH with MAU it was found out that the chance of identifying of hypofiltration in them in 2,2 times higher than in patients without MAU (59:94 vs. 13:46, odds ratio = 2.21). Researching GFR in 47 young patients with EAH with the experience of hypertension less than 3 years, hypofiltration was not noted in any patient, while at the same time hyperfiltration was detected in 21 (44,7%), most of them with MAU — 16 (76%). Thus MAU refers to the earliest markers of involvement in the pathological process of the kidneys in EAH. In the debut of EAH MAU is associated with hyperfiltration that develops apparently as a result of breakdown of the mechanisms of autoregulation of intrarenal blood flow, hypersecretion of locally-renal angiotensin II. As the HNP develops gradual decrease of GFR occurs. For the diagnosis of early stages of the HNP even a small decrease of GFR < 90 ml/min/1.73m2 without hypercreatininemia has diagnostic value.

2. Indicators of renal hemodynamics in patients with EAH depending on the presence and absence of MAU

Among 224 patients with EAH, who were performed Doppler research of intrarenal blood flow RI of interlobar renal arteries was significantly higher than among 57 healthy patients [respectively 0.65 (0.61; 0.675) and 0.6 (0.59; 0,61), U = 2366, p = 0.0000001]. The average RI was significantly higher in patients with EAH with MAU (138) — 0.66 (0.63; 0.69) than in patients with EAH without MAU (49) — 0.62 (0.59; 0.65), (U = 1576, p = 0.0000001). According to our data 40% of patients with EAH with MAU already have an increase in intrarenal vascular resistance (RI > 0.65) that indicates the probability of early development of hypertensive vascular nephropathy. In case of MAU probability of identifying of high RI was dependent on the total filtration function of kidney (Figure 1).

There is a chance to increase the intrarenal vascular resistance (RI > 0,65) among patients with hyperfiltration including those of military age with a short experience of hypertension and low blood pressure, but this chance is small — 1.54 ($\chi^2 = 0.07$, p= 0.9; in case of hypofiltration chance increases in 7 times ($\chi^2 = 11.03$,

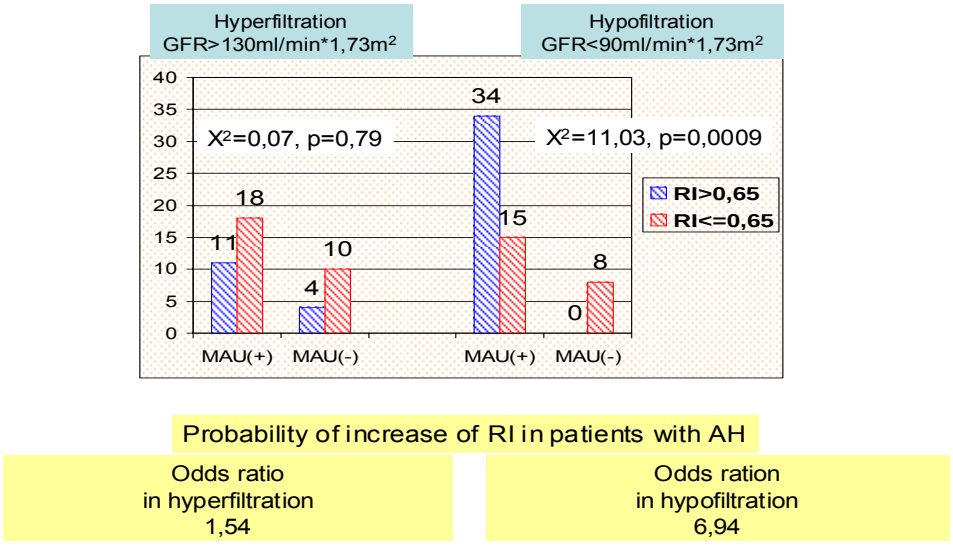

Figure 1.

p = 0.0009). Thus increasing of intrarenal vascular resistance (IRVR), measured by RI of interlobar renal arteries (> 0.65), is associated with MAU, the degree of GFR decrease, and therefore the increased RI can be considered as a marker for the further development of early GNP. Pathophysiological basis of increased resistance of renal artery is its hypertensive remodeling, in the development of which endothelial dysfunction and closely associated fibrogenesis play an important role.

3. Determination of urinary biomarkers of endothelial dysfunction and proteolysis / fibrinolysis and fibroangiogenesis in the kidney in patients with EAH

Comparative analysis of the test results of urinary biomarkers of proteolysis / fibrinolysis and fibroangiogenesis in the kidney in patients with EAH and the control group showed that the levels of all studied urinary biomarkers — PAI-1, TGF-β1 and VEGF, except for collagen of IV type, on average were significantly higher in patients EAH than in healthy individuals. Increase of the average level of collagen of IV type in the urine of patients with EAH were not significant (Table 2).

There is a strong positive correlation between the urinary excretion of collagen of IV type not only with MAU, but with RI (r = 0.73, p <0.01), which allows us to consider collagen of IV type secreted in the urine of patients with EAH along with high RI as indicators of late stage of GNP, which is characterized by activation of fibrogenesis in the kidney structures. By multivariable analysis of all studied markers of GNP we distinguish two factors combining 75% of studied parameters in the basic group of patients with EAH (Table 4). Factor 1, which combined MAU primarily with PAI-1 as well as TGF-β1 and VEGF, confirms that we identified the role of locally-renal endothelial dysfunction, the process of proteolysis / fibrinolysis and fibroangiogenesis in the development of the HNP. Factor 2, combined into one group RI and urinary excretion of collagen of IV type, reflects apparently the next stage of development of the HNP — maladaptive remodeling of the microvasculature due to increased renal fibrogenesis with accumulation of extracellular matrix in the vascular wall.

Table 2

Urinary excretion of PAI-I, TGF-β1, VEGF and collagen of IV type in patients with EAH and healthy individuals

Biomarker	n	Patients with EAH	n	Healthy individuals	Distinctions
PAI-1, ug / ml	53	0,176 (0,152;0,194)	12	0,147 (0,140;0,157)	U =104.5, p = 0.007
TGF-β_1, pg / ml	70	0,270 (0,225;0,297)	12	0,211 (0,2;0,223)	U = 110.5, p = 0.005
VEGF, pg / ml	71	81,33 (68,5;88,74)	12	52,68 (47,85;61,43)	U =69.5, p = 0.0001
Collagen of IV type, ng / ml	36	7,57 (3,21;15,5)	12	5,09 (2,15;6,4)	U = 86.5, p > 0.05

Table 3

Urinary excretion of the studied biomarkers — PAI-I, TGF-β1, VEGF, collagen of IV type in groups of patients with EAH with and without MAU

Biomarker	n	MAU (-)	n	MAU (+)	Distinctions
PAI-1, ug / ml	11	0,147 (0,130;0,161)	42	0,184 (0,165;0,197)	t=3.26, p=0.002
TGF-β_1, pg / ml	17	0,21 (0,193;0,237)	53	0,29 (0,244;0,306)	t=3.96, p=0.0002
VEGF, pg / ml	17	69,7 (64,1;78,42)	54	83,4 (73,15;90,73)	U=251, p=0.005
Collagen of IV type, ng / ml	10	3,07 (2,11;4,91)	26	10,3 (5,36;17,52)	U=55.5, p=0.008

In patients with EAH excretion of all the studied biomarkers including collagen of IV type depended on the presence of MAU — a key marker of early stages of GNP (Table 3).

We found a statistically significant direct correlation between MAU and the content of molecular mediators in the urine: PAI-1 (R = 0.53, p = 0.00004), TGF-β1 (R = 0.48; p = 0.0002), collagen of IV type (R = 0.43, p = 0.009) and VEGF (R = 0.36, p = 0.001), and also between the urinary excretion of PAI-1 and TGF-β1 (R = 0,37, p = 0,0066), TGF-β1 and VEGF (R = 0,42, p = 0.0003) confirming the activation of proteolysis / fibrinolysis and fibroangiogenesis in the kidney at early stage of the HNP. On the other hand, studied urinary biomarkers characterize the early stage of the HNP and can be used for its diagnosis.

Table 4

Factor analysis of the early signs of HNP-molecular mediators — PAI-I, TGF-β1, VEGF, collagen of IV type, MAU and RI

Biomarkers	Factor 1 (29%)	Factor 2 (46%)
RI	–0,02	–0,89
PAI-1	0,84	0,23
MAУ	0,80	–0,47
TGF-β_1	0,63	0,32
VEGF	0,67	0,52
Collagen of IV type	–0,11	–0,92

From clinical positions identifying persistent microalbuminuria along with following features – increased

urinary excretion of collagen of IV type, high resistance index in interlobar renal arteries (RI > 0.65) and trend toward decreasing of GFR (already less than 90 ml/min/1.73m2) in patients with EAH proves using of active nephroprotective strategy in these patients directed to prevention of impairment of renal function.

The presented data allow to conclude that the early stage of GNP is a dynamic process that has at every stage its clinical features and urinary biomarkers. The earliest marker of the HNP is microalbuminuria (MAU), which develops as a result of hemodynamic dysfunction caused by intrarenal vascular endothelial cells and the change in the total filtration function of kidney — early hyperfiltration with a gradual decrease in glomerular filtration rate for a long time without hypercreatininemia. At subsequent stages of the GNP on the background of persistent MAU and a trend toward hypofiltration we registered using Doppler method increase in resistance of intrarenal blood flow (RI > 0.65), which is confirmed by a direct link between MAU and RI, and feedback of MAU, and RI with degree of reduction of GFR.

Detection of MAU in patients with EAH is accompanied by increased urinary excretion of molecular mediators — PAI-1, TGF-β1 and VEGF, that on the one hand confirms the pathogenic role of urinary biomarkers in the assessment of endothelial dysfunction, the processes of proteolysis / fibrinolysis and fibroangiogenesis in the kidney during the HNP, on the other — suggests the possibility of using these urinary tests along with MAU for the diagnosis of early GNP. Moreover, our data shows that increased urinary excretion of mediators produced by endothelial cells can be detected in the urine of patients with EAH before the appearance of microalbuminuria. Clinical equivalents of maladaptive remodeling of intrarenal vessels in late stage of evolution of the early stages of GNP (in fact its progression) are increasing of the resistance index (RI > 0.65) at the level of the interlobar renal arteries, increased urinary excretion of collagen of IV type and tendency to decrease of GFR (< 90 ml/min/1.73m2).

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