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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

MARKERS FOR THE DIAGNOSIS OF PRECLINICAL KIDNEY DYSFUNCTION IN ARTERIAL HYPERTENSION AND ITS COMBINATION WITH TYPE 2 DIABETES MELLITUS

Speciality: 3205.01 – Internal Medicine
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OVERVIEW OF THE WORK

Relevance of the topic. A high incidence of damage to cardiovascular system is associated with an impaired renal function. A pronounced relationship between increased blood pressure (BP) and formation of any kidney pathology was identified, with the likelihood of its occurrence even with insignificant increases in BP[1]. Prognosis of arterial hypertension (AH) is largely determined by level of blood pressure and directly by kidney function, which is an independent marker of the occurrence of heart disease and mortality[2].

It is known that AH often occurs along with type 2 diabetes mellitus (DM). Diabetic nephropathy (DN) is identified in every second or third patient with type 2 diabetes approximately 5-7 years after the onset of the disease.

DN is characterized by significant changes in the structure of kidneys and constantly progressive complications[3].

At the same time, the specificity of negative changes in kidneys in type 2 DM is considered to be the absence of specific clinical signs at the beginning, which leads to delayed diagnosis[4].

The generally accepted criteria for nephropathy are slowing of the glomerular filtration rate (GFR) less than 60 ml/min and/or the presence of proteinuria. However, these criteria indicate a severe form of kidney damage, sometimes even irreversible renal pathology.

This is the main reason for the determined search for modern

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[1] Кобалава Ж.Д., Виллевальде С.В., Боровкова Н.Ю., Шутов А.М., Ничик Т.Е., Сафуанова Г.Ш. от имени исследователей программы ХРОНОГРАФ. Распространенность маркеров хронической болезни почек у пациентов с артериальной гипертонией: результаты эпидемиологического исследования ХРОНОГРАФ. Кардиология. 2017;57(10):39-44.

[2]Фуштей И.М., Подсевахина С.Л., Ткаченко О.В. и др. Факторы ухудшения функционального состояния почек у больных с артериальной гипертензией // Нефрология, 2016, №2 (64), с.128-131.

[3] Ian H. de Boer, Sripal Bangalore, Athanase Benetos, Andrew M. Davis, Erin D. Michos, Paul Muntner, Peter Rossing, Sophia Zoungas, George Bakris, Diabetes Care 2017 Sep; 40(9): 1273-1284.

[4] Colhoun H., Marcovecchio M. Biomarkers of diabetic kidney disease // Diabetologia, 2018, v.61, No 5, pp.996-1011.

and more accurate methods, in the application of which it will be possible to form an idea of kidney disorders in order to identify changes that indicate possibility of an intensive worsening of the course of chronic renal insufficiency[5].

All the aforementioned prompted us to conduct this study and determined our purposes and objectives.

The purpose of the research:

To study structural and functional changes in the kidneys of patients with AH as well as patients with AH along with type 2 DM; to determine laboratory and ultrasound markers of early renal dysfunction.

Research objectives:

1. To study the role of blood biochemical parameters reflecting renal function in the detection of early renal damage in patients with AH and in patients with AH along with type 2 DM;
2. To calculate the glomerular filtration rate by serum creatinine and cystatin C in patients with AH and patients with AH along with type 2 DM;
3. To identify the degree of albuminuria in patients with AH and patients with AH along with type 2 DM;
4. To conduct ultrasound examination with Doppler ultrasonography of the renal blood flow in order to assess biometric parameters of kidneys and blood supply parameters in patients with AH and patients with AH along with type 2 DM;
5. To identify sensitive markers for preclinical diagnosis of structural and functional abnormalities in kidneys of patients with AH and patients with AH along with type 2 DM.

Scientific novelty.

- A complex of markers including concentration of cystatin C and uric acid, GFR value, albuminuria degree and ultrasound data was used to study early renal dysfunction in patients with AH and patients with AH along with type 2 DM;

[5]Katherine G., William B., George L. et al. Kidney Biomarkers and Decline in EGFR in patients with type 2 Diabetes // Clinical Journal of the American Sosiety of Nephrology, 2018, v.13, No 5, pp. 23-27.

- A comparative assessment of indicators was carried out in order to identify more informative markers for the detection of preclinical changes in kidneys of patients with AH of I-II degrees and patients with AH along with type 2 DM;
- A diagnostic value of cystatin C in AH and AH along with type 2 DM, was investigated as compared with traditional research methods.

Practical significance. A complex of studies, including diagnosis of cystatin C levels, level of albuminuria in addition to the ultrasound data in patients with AH and patients with AH along with type 2 DM, makes it possible to detect renal dysfunction at an early stage and thereby optimize the treatment strategy by preventing or reducing number of nephrological and cardiovascular complications.

Main points submitted for the thesis defense:

- When assessing blood parameters reflecting functional position of kidneys both in AH and AH along with type 2 DM, a quantitative assessment of cystatin C makes it possible to detect the degree of renal damage at an earlier stage in comparison with traditional indicators (urea, creatinine, residual nitrogen);
- When assessing early renal dysfunction, in addition to laboratory examination of cystatin C level, calculation of GFR by its concentration is relatively more sensitive and informative;
- A decrease in the linear velocity of renal blood flow and an increase in vascular resistance in patients with AH and patients with AH along with type 2 DM, as well as an increase in renal volume in AH along with type 2 DM, together with laboratory data, signify early renal dysfunction;
- A complex of mutually confirming studies conducted for the assessment of functional state of kidneys, which includes biochemical blood analysis, calculation of GFR, determination of albuminuria degree, ultrasonography along with renal blood flow assessment, allows detecting kidney damage in the preclinical stage.

Implementation of research results. The results of the study were introduced into the practical work of I and II cardiology departments of the Research Institute of Cardiology named after acad. J. Abdullayev, Department of Therapeutic and Pediatric Propedeutics of Azerbaijan Medical University and Department of Nephrology and Urology of the Educational and Therapeutic Clinic of Azerbaijan Medical University.

The initial discussion of the thesis was held at the Academic Council of the Scientific Research Institute of Cardiology named after acad. J. Abdullayev on May 21, 2018 (protocol №4).

The scientific seminar of the one-time council BFD 2.27/4 created on the basis of the Dissertation Council ED 2.27 operating at Azerbaijan Medical University was held on June 22, 2021(protocol №6).

Publications. 10 papers were published based on the results of the dissertation including 7 scientific articles (5 within the republic, 2 abroad), 3 theses (2 within the republic, 1 abroad). 4 scientific articles without co-authors.

Structure and length. The dissertation is printed on 146 pages and consists of introduction, 3 chapters, discussion, conclusion and practical recommendations. The list of references contains 213 sources (5 internal and 208 foreign). The paper contains 8 figures and 23 tables.

MATERIALS AND RESEARCH METHODS

100 patients aged 35-58 years (median age 46.8 ± 0.68) were examined. The patients were divided into 2 groups. The first group included 50 patients with AH, the second group included 50 patients with AH along with type 2 DM. The 1st group was also divided into 2 subgroups: the first subgroup (IA) consisted of 22 patients with AH of I degree, the second subgroup (IБ) included 28 patients with AH of II degree. To compare the results, a control group of 30 practically healthy people aged 44.5 ± 1.11 years (15 men, 15 women) with a normal blood pressure level was also included in the study.

The recommendations of the European Society of Hypertension

(ESH) and the European Society of Cardiology (ESC) were used for the diagnosis of hypertension. Patients with symptomatic high blood pressure, chronic cardiac insufficiency, history of myocardial infarction, chronic lung and kidney disease were not included in the study.

All patients underwent general clinical and laboratory-instrumental examination using anthropometric parameters.

The subjects underwent general and biochemical blood tests, in addition the levels of their creatinine (by the Jaffe reaction, on the MindrayBA-88A" analyzer), urea, uric acid (by the turbidimetric method), cystatin C (by the enzyme-linked immunosorbent assay (Elisa) on the Mindray-MR- 96A"), as well as blood lipid profile (photometric method on a photometer), glucose and glycated hemoglobin levels were identified.

Calculation of creatinine clearance (CrCl) was performed using the Cockroft – Gault formula, GFR was calculated using the abbreviated formula MDRD, CKD-EPI and the Hoek formula based on cystatin.

Below 30 mg/g were taken as normal values. The indicators from 30 to 300 mg/g confirmed in 2 of 3 quantitative analyzes, with a time interval of 7-10 days, in the absence of a rise in body temperature and symptoms of urinary tract infection, were regarded as albuminuria category A2.

As a general rule, all patients underwent kidney ultrasound using a Mindray DC-N6 apparatus with a 3.5 MHz convex probe. The linear dimensions of kidneys were determined: longitudinal dimension in the frontal plane (L), thickness (T) and height (H) of the kidneys in the plane of transverse and longitudinal axes. In addition, thickness of the parenchyma (P) of the kidneys was determined. Volumes of the kidneys (V) were calculated according to the formula proposed by H. Hricak: $V=0.53\times L\times H\times T$; where 0.53 is the coefficient. Kidney shape index (J) was also calculated: $J=L/H+T$

The degree of albuminuria was determined using microalbu-FAN test kits and the values

Pulse-wave Doppler sonography was used to determine peak systolic blood flow velocity (Vs), end diastolic blood flow velocity

(Vd), resistance index (RI), and pulsation index (PI).

Preclinical kidney damage was assessed by the degree of albuminuria, concentration of creatinine, uric acid, cystatin C in the blood, calculated GFR, and renal ultrasound data.

According to the purpose and objectives of the study, the results obtained were processed by statistical methods. To characterize a group of homogeneous units, their arithmetic mean values (M) and their standard errors (m) were determined. Qualitative signs in the groups were characterized by their absolute number in each group and its share expressed as a percentage (%).

To compare the data between the groups obtained during the study, the Mann-Whitney U-test, a nonparametric method for assessing the differences between two independent samples, was used. The statistical difference was considered significant at $p < 0.05$.

Correlation analysis of the data was used to identify the relations between clinical and biochemical parameters and to identify the degree and direction of the relations between the values. For this purpose, the linear r-Pearson correlation coefficient was found.

Statistical analysis of the research results was carried out on a personal computer using modern software programs: Microsoft Excel spreadsheet software and a software program for statistical data analysis IBM SPSS Statistics 22 (Statistical Package for the Social Sciences).

The recommendations of G.P. Kotelnikov and A.S. Shpigel were used in order to determine the effectiveness and specificity of the studies carried out.

RESULTS AND DISCUSSION

When comparing biochemical parameters of blood in patients of the study groups, the level of creatinine remained within the normal range (Table 1). In the IA subgroup patients with AH of I degree, the median creatinine level was 72.4 ± 1.64 μmol/l. Moreover, its maximum concentration in this subgroup was 83.5 μmol/l. When compared to the control group, there was no statistically significant difference ($p > 0.05$). In the control group, in practically healthy

individuals, this indicator was 71.1 ± 0.64 μmol /l, without exceeding 80.0 μmol /l.

In the IB subgroup (patients with AH of II degree) the median creatinine value was 78.1±2.09 μmol/l. Despite the fact that the concentration of creatinine in this study group was within the normal range, the results obtained significantly differed from the control group ($p<0.01$).

Table 1

Comparative assessment of biochemical parameters of blood, reflecting renal function in patients with AH and patients with AH along with type 2 DM (M±m)

Indicators / Groups	Creatinine, μmol/l	Uric acid, μmol/l	Urea, mmol/l	Cystatin C, mg/l
Control group, n=30	71,1±0,64 (66,0-80,0)	278,0±12,4 (160,0-360,0)	4,51±0,20 (2,8-7,2)	0,75±0,008 (0,65-0,84)
IA subgroup n=22	72,4±1,64 (60,0-83,5)	415,0±6,65 (348,0-460,0)	4,56±0,18 (3,23-6,83)	0,84±0,014 (0,59-0,95)
$P_к$	>0,05	<0,001	>0,05	<0,001
IБ subgroup n=28	78,1±2,09 (58,0-114,0)	422,9±5,50 (336,0-473,0)	4,80±0,16 (3,1-7,2)	1,09±0,042 (0,58-1,3)
$P_к$	<0,01	<0,001	>0,05	<0,001
2 nd group n=50	86,5±1,94 (70,0-120,0)	439,6±4,0 (350,0-480,0)	5,32±0,18 (3,6-8,35)	1,14±0,032 (0,8-1,48)
P_c	<0,001	<0,001	<0,01	<0,001
P_{IA}	<0,001	<0,01	<0,01	<0,001
$P_{IБ}$	<0,01	<0,05	<0,05	>0,05

Note: P_c - statistical significance of indicators compared to the control group; P_{IA} - statistical significance of indicators compared to the IA group; $P_{IБ}$ - statistical significance of indicators compared to the IБ group.

In the 2nd group, the median creatinine value was 86.5±1.94 μmol/l, which was within the normal range, but also statistically significantly different from the control group ($p < 0.001$).

In the same group, the highest creatinine value was 120 μmol/l, which considerably differed from the control group, as well as the IA ($p<0.001$) and IБ subgroups ($p<0.01$).

Creatinine is characterized as a product of muscle tissue metabolism, and therefore its concentration is associated with the volume of muscle mass and, depending on this mass, it is subject to insignificant daily fluctuations. In addition, considerable changes in its level are possible over a long period of time, when there is a significant change in body weight. On the other hand, along with glomerular filtration, there is also a tubular secretion of creatinine, which increases with a decrease in renal function.

And finally, determination of the concentration of serum creatinine does not mean that it is a highly sensitive method, since only after a 50% decrease in glomerular filtration, the amount of creatinine begins to respond with an increase[6].

Considering all of the above, along with determination of creatinine concentration in the blood, we studied the cystatin C marker, which is more valuable and significant in prognostic terms for detecting early kidney damage[7]. When studying the quantitative level of cystatin C in blood, in contrast to creatinine, its increase was observed even with AH of I degree with a median value of 0.84±0.014 mg/l (Table 1). In this subgroup, the highest value of cystatin C was 0.95 mg/l. Considering the normal value of this indicator, significant differences were revealed between the IA subgroup and the control group ($p < 0.001$).

An increase in the level of cystatin C was also observed in the IБ subgroup reaching the median value of 1.1±0.042 mg/l. In this

[6] Правника Е.А. К проблеме определения функции почек у пациентов с гипертонической болезнью (литературный обзор) // Сибирский научно-медицинский журнал, 2014, №6, с.1-12.

[7] Исакова А.С. Цистатин как маркер нарушения фильтрационной функции почек при кардиоренальном синдроме // Вестник КазНМУ 2013, №4 (1), с.318-320.

subgroup, the cystatin C concentration varied in the range of 0.58-1.3 mg/l. At the same time, the result obtained was significant in comparison with both the control group and the IA subgroup ($p < 0.001$).

In the 2nd group, the level of cystatin C in patients with AH along with type 2 DM, averaged 1.14±0.03 mg/l. The cystatin C level in this group changed in the range of 0.8-1.48 mg/l. This indicator significantly differed from the control group ($p < 0.001$) and the IA subgroup ($p < 0.001$). On the other hand, it was not considerably different from the IB subgroup.

The urea level in the 1st and 2nd groups did not differ from the control group (Table 1). Indicators of urea in the IA subgroup averaged 4.56±0.18 mmol.

In the IБ subgroup, its median concentration was 4.80 ± 0.16 mmol/l, which was within the normal range. When comparing these values with the data of the control group, no statistically significant difference was found ($p > 0.05$). Considering the predictive value of uricemia, a quantitative analysis of uric acid in blood was also performed. Information on the importance of uric acid is common in the literature.

Thus, it is noted that significant changes in the level of uricemia in patients with AH indicate a greater likelihood of progression of hypertensive nephropathy, which coincided with our results[8]. According to our data, the average level of uricemia in the IA and IB subgroups was 415.0±6.65 mmol/l and 422.9±5.50 mmol/l, respectively. In the 2nd group, the average value was 439.6±4.0 mmol/l (Table 1).

The use of calculation methods in order to assess renal function is more correct than calculating only serum creatinine concentration. Experience of using calculation methods shows that a subclinical

[8] КобалаваЖ.Д., Троицкая Е.А. Бессимптомная гиперурикемия: подходы к лечению в аспекте риска развития сердечно-сосудистых и почечных заболеваний // Кардиология, 2020, №12, с.104-109 Фуштей И.М., Подсевахина С.Л., Ткаченко О.В. и др. Факторы ухудшения функционального состояния почек у больных с артериальной гипертензией // Нефрология, 2016, №2 (64), с.128-131.

increase in serum creatinine may not be accompanied by a decrease in GFR <60 ml/min/1.73 m[29]. Considering this, the GFR calculated by various formulas was also studied to assess renal function in patients with AH and patients with AH along with type 2 DM (Fig. 1).

When calculating creatinine clearance in the patients of the 1st group using the Cockroft-Gault formula, no statistically significant differences were found between the subgroups (Fig. 1). The creatinine clearance in the IA subgroup averaged 104.9±2.09 ml/min, which did not statistically differ from the control group, where this indicator was within 101.4±1.74 ml/min. The maximum creatinine clearance in this subgroup was 128.9 ml/min, which corresponded to hyperfiltration.

In the IБ subgroup this value did not differ from the IA subgroup and averaged 102.1±3.35 ml/min, which was insignificant compared to the control group.

There was a noticeable decrease in the creatinine clearance, averaging 94.3±2.25 ml/min, in the patients with AH and the patients with AH along with type 2 DM, in contrast to the group of the patients without DM. The lowest value in this group was 60.6 ml/min, and the highest was 121.3 ml/min. Also, the result obtained in this group was significant compared to the control and IA subgroups ($p<0.001$). However, no difference was found when compared with the IB subgroup ($p>0.05$).

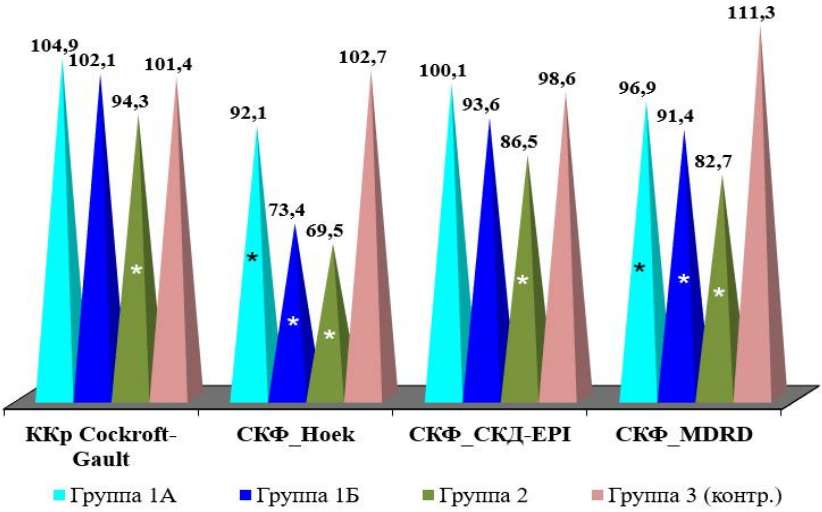
When calculating GFR using the MDRD formula in the IA subgroup patients, the result was statistically significant in comparison with the control group, averaging 96.9±2.04 ml/min/1.73 m^2 ($p<0.001$). The highest value obtained when calculating the GFR using this formula, as well as when determining the creatinine clearance was 128.9 ml/min/1.73 m^2.

The GFR calculated using the MDRD formula in the patients of the IБ subgroup averaged 91.4±3.31 ml/min/1.73 m^2, which statistically significantly differed from both the control group and the IA subgroup.

[9] Батюшин М.М. Методические основы оценки скорости клубочковой фильтрации в урологической практике // Вестник урологии, 2017, №1, том 5, с.42-51.

The GFR calculated using the MDRD formula in the patients with AH along with type 2 DM was reduced by the average of 82.7±2.18 ml/min/1.73 m^2. The lowest GFR in this group was 51 ml/min/1.73 m^2, while the highest was 111.5 ml/min/1.73 m^2. The results obtained in this group were statistically significant compared to the IA subgroup ($p < 0.001$).

It was noticeable that when calculating the GFR using the MDRD formula, there was a more significant decrease in renal function in the patients with AH along with type 2 DM compared to the patients without DM.



* - the indicator is statistically reliably different from the control group, $p < 0.05$

Fig. 1 The GFR, calculated by various formulas in the patients with AH and the patients with AH along with type 2 DM

The GFR values obtained using the CKD EPI formula were also considered. The GFR value in the IA subgroup, being within the normal range (on average 100.1±1.27 ml/min/1.73 m^2), was lower compared with the creatinine clearance. The GFR in this subgroup did not statistically differ from the control group, where it averaged

98.6±1.04 ml/min/1.73 m^2 (p> 0.05).

The GFR calculated using this formula in the patients with AH of I degree varied in the range of 92.4-119.8 ml/min/1.73 m^2. When calculated using the CKD EPI formula, there was a statistical difference between the subgroups (p <0.05).

In the patients with AH of II degree, the GFR value calculated using the CKD EPI formula averaged 93.6±2.70 ml/min/1.73 m^2, which did not statistically differ from the control group (p> 0.05). In this subgroup, the GFR value varied from 60 to 116.8 ml/min/1.73 m^2.

As can be seen, the GFR values obtained by the MDRD and CKD EPI formulas in all groups were lower in comparison with the creatinine clearance.

In the calculations of the GFR using various formulas, the pattern of changes in glomerular filtration was analyzed. Based on the data obtained among the AH patients of the IA subgroup the hyperfiltration by creatinine clearance was observed in 2 patients, and the GFR was higher than 120 ml/min. When calculated using the Cockroft-Gault formula, the normal value of the creatinine clearance in this group was detected in 20 patients. At the same time, no decrease in the clearance below 60 ml/min was observed. In 1 patient with AH of I degree, the GFR calculated using the MDRD formula was also changed towards hyperfiltration.

Hyperfiltration was not detected in this subgroup when calculated using the CKD EPI. The values obtained as a result of the GFR calculations in other patients were within the normal range of 90-120 ml/min/m^2.

Among the patients in the 2^{nd} group (patients with AH along with type 2 DM) a change in the creatinine clearance was found in 15 patients. At the same time, in 2 patients these changes were found in the form of hyperfiltration, and in 13 patients in the form of hypofiltration being in the range of 60-90 ml/min. Hypofiltration in 13 patients was expressed by a slight decrease in the GFR.

The change in the GFR calculated using the MDRD formula was found in 21 patients of the 2nd group. The GFR values obtained revealed hypofiltration. Moreover, in 4% of cases, a moderate decrease in renal function was found (GFR <60 ml/min/1.73 m^2),

corresponding to 3rd stage of chronic kidney disease (CKD).

In 32 patients with AH along with type 2 DM, the GFR, when calculated using the CKD EPI formula, was within the normal range. A slight decrease in the GFR by the CKD EPI formula, ranging from 60-90 ml/min/1.73 m^2, was found in 16 patients. Using this formula, just like the MDRD formula, in 4% of cases, a moderate decrease in renal function was revealed, in which the GFR value fell below 60 ml/min/1.73 m^2.

We also compared the formulas by the number of violations found. When comparing groups, the use of the Cockroft-Gault formula revealed a greater number of patients (28%) with hyperfiltration among the patients with AH of II degree, while hypofiltration prevailed in the 2nd group, where one patient had the GFR below 60 ml/min.

When calculating the GFR using the MDRD formula, hyperfiltration was detected in the patients with AH of I and II degree, in contrast to the patients of the 2nd group. A decrease in the GFR <60 ml/min/1.73 m^2 was found in 2 patients of the 2nd group, while in the 1st group such changes were not observed. The data obtained as a result of the GFR calculations using the CKD EPI formula were close to those obtained using the MDRD formula.

Thus, when assessing renal dysfunction using the GFR calculations, the use of the Cockroft-Gault formula revealed a greater number of patients with hyperfiltration. The maximum number of patients with a diagnostically significant (<60 ml/min/1.73 m^2) decrease in the GFR was found in the patients with AH along with type 2 DM, when calculated using both the MDRD and CKD EPI formulas.

It should be noted that the MDRD formula is recommended to be used only when the GFR values are less than 90 ml/min/1.73 m^2. In cases when the GFR values are higher, this formula is inappropriate to use due to the lack of a relevant research evidentiary base[10].

For a more accurate assessment of renal function, the GFR was

[10] Levey AS, Tighiouart H, Simon AL, Inker LA. Comparing Newer GFR Estimating Equations Using Creatinine and Cystatin C to the CKD-EPI Equations in Adults. Am J Kidney Dis 2017; 70:587.

also calculated based on the concentration of cystatin C using the Hoek formula. When calculating the GFR by the level of cystatin C in the IA subgroup patients, the median value was 92.1±2.01 ml/min/1.73 m^2. The range of changes in the value of the calculated GFR in this subgroup varied from 80.3 to 131.9 ml/min/1.73 m^2. The result obtained was significantly different from the control group ($p < 0.001$).

In patients with AH of II degree, the GFR calculated by cystatin C was on average 73.4±4.19 ml/min/1.73 m^2, while the minimum and maximum values were 57.5 ml/min/1.73 m^2 and 134.2 ml/min/1.73 m^2, respectively. In comparison with the control group, the result obtained was significantly decreased ($p < 0.001$). A statistically significant difference was also observed when comparing GFR between subgroups.

In the patients with AH along with type 2 DM, a decrease in the GFR to an average value of 69.5±2.26 ml/min/1.73 m^2 was observed. This group had the lowest GFR of 50 ml/min/1.73 m^2. The revealed changes significantly differed from both the control and the 1st group ($p < 0.001$).

When calculating the GFR by the level of cystatin C in the control group (practically healthy individuals), its average value was 102.7±1.16 ml/min/1.73 m^2, while the range of changes varied from 91.3 to 119.3 ml/min/1.73 m^2.

When assessing the GFR calculated from the concentration of cystatin C by the Hoek formula in the IA subgroup, renal dysfunction was revealed in 3 patients with AH of I degree.

At the same time, hyperfiltration was observed in 1 patient, with an increase in the GFR of more than 120 ml/min/1.73 m^2. While there was a slight decrease in renal function of 2 patients corresponding to the GFR range from 60 to 90 ml/min/1.73 m^2.

In the IB subgroup, when calculating the GFR using the Hoek formula, based on the concentration of cystatin C, hyperfiltration was observed in 3 patients. In the same subgroup, hypofiltration was revealed in 21 patients. 19 of them had hypofiltration in the form of slightly reduced renal function (GFR range 60-89 ml/min/1.73 m^2), and the GFR of 2 patients was below 60 ml/min/1.73 m^2.

When calculating the GFR based on the concentration of cystatin C in the patients with AH along with type 2 DM, 7 patients showed a decrease in the GFR of less than 60 ml/min/1.73 m^2. In 25 patients, the GFR decreased to values corresponding to the preclinical stage of kidney damage, which is in the range of 60-89 ml/min/1.73 m^2.

Comparison of the GFR values by the concentration of cystatin C between the 1st and the 2nd groups revealed the prevalence of cases of kidney dysfunction in patients with AH along with type 2 DM.

Comparative assessment of GFR calculated from the level of cystatin C and other formulas based on the level of creatinine revealed the largest decrease in GFR when calculated using the Hoek formula (Fig. 1).

The results demonstrated that with a decrease in the GFR from 120 to 60 ml/min/1.73 m^2, the concentration of cystatin C in the blood serum increased more clearly in comparison with creatinine.

When calculating the GFR by creatinine levels, a smaller number of patients with a slightly decreased GFR (range 60-89 ml/min/1.73 m^2) were detected. GFR <60 ml/min/1.73 m^2, calculated from the creatinine level, was detected only in 2 patients with AH along with type 2 DM.

The results obtained showed that the determination of the GFR by the level of cystatin C, in contrast to the calculation by creatinine, is more appropriate, which coincides with the data of other researchers.

According to the results on the albuminuria degree in the form of the albumin/creatinine ratio, the patients with AH without type 2 DM included in the study were divided into two categories - with albuminuria of A2 gradation and albuminuria of A1 gradation. In the patients with AH along with type 2 DM, there were also cases with A3 albuminuria (Fig. 2).

The average value of A2 albuminuria in the IA subgroup was 37.1 ± 2.68 mg/g, and in the IB subgroup it was 58.7 ± 2.36 mg/g.

When considering the results obtained, based on the digital values of BP, it was revealed that among the subjects with AH of I degree, albuminuria A2 was detected in 2 patients. The rest of the

patients in this group had A1 grade albuminuria.

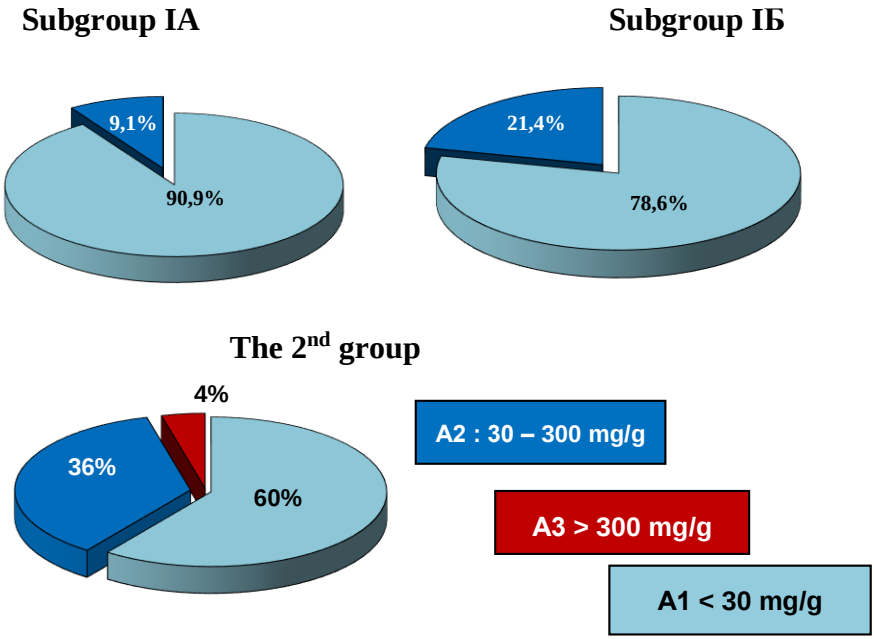


Fig. 2 Frequency of the occurrence of albuminuria categories in the patients with AH of I and II degrees, as well as the patients with AH along with type 2 DM

Albuminuria A2 was observed in 18 patients with AH along with type 2 DM. The average level of albuminuria in this group was 62.3 ± 7.30 mg/g. In the same group, 2 patients had A3 albuminuria. Category A1 was found in 30 patients.

It should be noted that albuminuria A2 was detected in 9.1% of the patients with AH of I degree, in 21.4% of the patients with AH of II degree and in 36% of the patients with AH along with type 2 DM. In the patients with AH along with type 2 DM, albuminuria of grade A3 was detected in 4% of cases, when the albumin/creatinine ratio was higher than 300 mg/g.

Along with determining the concentration of creatinine and cystatin C, calculating the GFR based on them, identifying the degree

of albuminuria, a correlation analysis of the results was carried out. A correlation between cystatin C level and the concentration of creatinine was identified in all groups. Both in the IA and IБ subgroups, the correlation was moderate and weak corresponding to r = 0.313 (p> 0.05) and r = 0.236 (p> 0.05) respectively, and at the same time it was statistically unreliable. There was a moderate correlation between the level of creatinine and cystatin C (r = 0.291, p <0.05) in the second group. In the examined patients, the relationship between cystatin C and GFR calculated using various formulas was observed in the following order.

The level of cystatin C was moderately inversely correlated with the CCr (r = -0.400, p <0.05) in the IA subgroup. While there was a weak inverse correlation (r = -0.250, p> 0.05) with the GFR calculated using the MDRD formula, as well as moderate inverse correlation (r = -0.350, p> 0.05) with the GFR calculated using the CKD-EPI formula.

In the IБ subgroup, the concentration of cystatin C also moderately negatively correlated with creatinine clearance (r = -0.413, p <0.05) and moderately inversely with GFR calculated using the MDRD (r = -0.324, p> 0.05) and the CKD-EPI formulas (r = -0.326, p> 0.05). A similar result was noted in the second group, where a weak negative correlation was revealed based on three formulas, with r = -0.325 (p <0.05), r = -0.229 (p> 0.05), r = -0.229 (p> 0.05), respectively. Compared to the creatinine concentration, the cystatin C value did not correlate with albuminuria in the IA and IБ subgroups. Whereas, in the second group, a weak correlation was found between the level of cystatin C and the excretion of albumin (r = 0.257, p> 0.05). This indicates that both albuminuria and cystatin C levels are independent indicators of renal dysfunction.

The nature of structural damage to the kidneys in the AH patients, as well as in the patients with AH along with type 2 DM has been reflected in the literature to a lesser extent. These features are based, in particular, on the results of pathological studies.

The nature of structural damage to the kidneys in hypertension, as well as in the adjacent course of type 2 diabetes in the literature is reflected to a lesser extent. These features are based, in particular, on

the results of pathomorphological studies of kidney biopsies. An increase in the volumes of the kidneys of more than 184.16±22.49 cm^3 and a decrease in the index of the shape of the kidneys less than 1.01±0.07 cm^3 are considered as predictors of structural changes in the kidneys[11]. When determining the biometric parameters of the kidneys in the patients with AH of I degree, the average kidney length corresponded to 10.7±0.12 cm, height to 5.15±0.04 cm and thickness to 4.5±0.04 cm (table 2). As can be seen in the patients with AH of I degree, these indicators corresponded to normal values. Therefore, there was no statistical difference between the IA subgroup and the control group for this indicator ($p > 0.05$).

The average value of kidney volumes in this group was 131.9±1.88 cm^3, which did not differ statistically from the control group ($p > 0.05$). The volume of the kidneys varied in the range of 118.9 - 149.9 cm^3.

When studying the thickness of the parenchyma (P), the obtained values were within the normal range, equal to 1.69±0.019 cm, which did not statistically differ from the control group ($p > 0.05$).

In practically healthy individuals, the thickness of the renal parenchyma averaged 1.72±0.015 cm.

In the patients of the IБ subgroup (with AH of II degree), the biometric parameters of the kidneys did not differ from the IA subgroup. The length was 10.9±0.09 cm, the height was 5.15±0.05 cm and the thickness was 4.59±0.04 cm.

The kidney volume in these patients was also within the normal range, averaging 137.0±2.29 cm^3. The largest volume was 168.0 cm^3.

The index of the shape of the kidneys in patients with AH of I and II degrees did not change. The thickness of the renal parenchyma in this group was also within the normal range, not differing from the control group.

In the patients of the 2nd group, the linear indicators of the kidneys were as following: the length averaged 12.6±0.06 cm, the

[11] Капустин С.В., Оуен Р., Пиманов С.И. Ультразвуковое исследование в урологии и нефрологии. Монография / – 2-е изд., испр. и доп. – М.: Умный доктор, 2017. - 176 с.

height was 5.84±0.04 cm and the thickness was 5.76±0.03 cm, which was statistically significant when compared to the control group and the 1st group of patients. The volumes of the kidneys of the patients of this group were in the range of 180.5 - 264.4 cm^3.

Table 2

Comparative assessment of biometric parameters of the kidneys according to ultrasound data in the patients with AH of I and II degrees and the patients with AH along with type 2 DM (M ± m)

Indicators / Groups	Length L, sm	Height H, sm	Thickness T, sm	Volume V, sm^3
Control group, n=30	10,8±0,08 (9,4-11,4)	5,29±0,06 (4,6-5,7)	4,36±0,04 (4,1-5,0)	133,1±2,88 (94,0-167,7)
IA subgroup n=22	10,7±0,12 (9,6-11,6)	5,15±0,04 (4,8-5,4)	4,50±0,043 (4,2-4,9)	131,9±1,88 (118,9-149,9)
P_c	>0,05	<0,05	<0,05	>0,05
IБ subgroup n=28	10,9±0,09 (9,8-11,7)	5,15,±0,05 (4,7-5,5)	4,59±0,04 (4,3-5,1)	137,0±2,29 (109,9-168,0)
P_c	>0,05	<0,05	<0,05	>0,05
2nd group n=50	12,6±0,06 (11,7-13,2)	5,84±0,04 (5,3-6,3)	5,76±0,03 (5,4-6,0)	224,6±3,17 (180,5-264,4)
P_c	<0,001	<0,001	<0,001	<0,001
P_{IA}	<0,001	<0,001	<0,001	<0,001
$P_{IБ}$	<0,001	<0,001	<0,001	<0,001

Note: P_c - statistical significance of indicators compared to the control group; P_{IA} - statistical significance of indicators compared to the IA group; $P_{IБ}$ - statistical significance of indicators compared to the IБ group

The thickness of the parenchyma in the patients with AH along with type 2 DM increased. A characteristic significant increase in the average volume of the kidneys of the patients in this group was

noticeable (224.6±3.17 cm^3) compared to the control group (133.1±2.88 cm^3) ($p < 0.001$).

The results obtained in the course of the study, coinciding with the data of other studies, show that the renal volume in the patients with DM is significantly greater than in healthy people.

We also studied the renal blood circulation using Doppler ultrasound test. The following results were obtained in the study of intrarenal blood supply in the patients of the IA and IB subgroups at the level of segmental arteries with an assessment of quantitative characteristics (Table 3).

In the patients of the IA subgroup, the average value of the maximum blood flow velocity (Vs) in the segmental arteries of the kidneys was 0.53 ± 0.006 m/s, and the minimum blood flow velocity (Vd) averaged 0.20 ± 0.004 m/s. Both indicators were statistically significantly different from the control group ($p < 0.01$). In the control group (practically healthy individuals), the indicator of the maximum blood flow velocity averaged 0.58 ± 0.006 m/s, and the minimum blood flow velocity was 0.23 ± 0.003 m/s.

When assessing vascular resistance, the average value of the resistance index (RI) in this group was 0.62 ± 0.008, and the pulsation index (PI) was 0.78 ± 0.017. There was no significant difference in the relative values of blood supply between the control group and the patients of the IA subgroup ($p > 0.05$).

The inear indicators of intrarenal blood flow velocity in the patients with AH of II degree (Vs: 0.46 ± 0.006 m/s; Vd: 0.17 ± 0.002 m/s) were statistically significantly lower than in the control group ($p < 0.001$). The maximum blood flow velocity of these patients averaged 0.46 ± 0.006 m/s, and the minimum blood flow velocity was 0.17 ± 0.002 m/s, which indicates a decrease in renal blood flow in this group as well.

The indicators for of the resistance index and pulsation index in the patients with AH of II degree also exceeded those for the control group, which indicates a higher intrarenal vascular resistance in the patients with AH of II degree. The RI averaged 0.64 ± 0.006. The range of fluctuations of this indicator was between 0.58 and 0.70, which was statistically significant compared with both the control

group and the IA subgroup.

Table 3

The state of renal blood flow in the patients with AH and with AH along with type 2 DM

(M ± m)

Indicators / Groups	Vs m/s	Vd m/s	RI	PI
Control group, n=30	0,58+0,006 (0,52-0,62)	0,23+0,003 (0,20-0,25)	0,60+0,006 (0,53-0,67)	0,80+0,015 (0,63-0,95)
IA subgroup n=22	0,53+0,006 (0,47-0,60)	0,20+0,004 (0,16-0,24)	0,62+0,008 (0,54-0,67)	0,78+0,017 (0,60-0,90)
P_c	<0,01	<0,01	>0,05	>0,05
IБ subgroup n=28	0,46+0,006 (0,41-0,51)	0,17+0,002 (0,13-0,18)	0,64+0,006 (0,58-0,70)	0,85+0,018 (0,71-1,01)
P_c	<0,001	<0,001	<0,01	<0,05
P_{IA}	<0,001	<0,01	<0,05	<0,01
2nd group n=50	0,39+0,003 (0,36-0,42)	0,13+0,002 (0,10-0,16)	0,67+0,006 (0,58-0,75)	0,92+0,011 (0,77-1,08)
P_c	<0,001	<0,001	<0,001	<0,001
P_{IA}	<0,001	<0,001	<0,001	<0,001
$P_{IБ}$	<0,001	<0,01	<0,01	<0,01

Note: P_c - statistical significance of indicators compared to the control group; P_{IA} - statistical significance of indicators compared to the IA group; $P_{IБ}$ - statistical significance of indicators compared to the IБ group

The PI in this group averaged 0.85 ± 0.018 with the range of fluctuations from 0.71 to 1.01.

In a quantitative analysis of hemodynamic parameters of intrarenal blood flow in the 2nd group, the following parameters were noted: the average value of the maximum blood flow velocity in the kidneys was 0.39 ± 0.003 m/s, while the minimum blood flow velocity was 0.13 ± 0.002 m/s (p <0.001). The results obtained were statistically significantly different from the 1st group and the control group. In the patients with AH along with type 2 DM, the greatest decrease in linear velocities was revealed. The smallest value of the maximum blood flow velocity was 0.36 m/s, and the minimum blood

flow velocity was 0.10 m/s.

The average value of the peripheral resistance index: the RI was equal to 0.67 ± 0.006, and the PI was 0.92 ± 0.011 ($p < 0.001$). The range of variations in the resistance index was between 0.58 and 0.75, and the pulsation index was between 0.77 and 1.08.

As can be seen from the results obtained, despite the change in linear blood flow velocities in the patients with AH along with type 2 DM, more significant changes were found in comparison with the AH patients without diabetes.

Changes in the biometric parameters of the kidneys with impaired renal blood flow are associated with functional changes and reflect the process of kidney damage both in the patients with AH and the patients with AH along with type 2 DM.

Thus, the data of ultrasound examination with Doppler ultrasonography of the renal blood flow, along with the indicators of laboratory tests, presented specific diagnostic markers of early renal dysfunction in the patients with AH and the patients with AH along with type 2 DM, and their sensitivity was 61.7%.

Considering the literature data and our study, it should be noted that there is a sufficient amount of convincing data on the need for scrupulous monitoring of renal function in individuals at risk of CKD, including patients with AH, DM, on the importance of timely monitoring of patients with a mild form of renal dysfunction. However, for an objective assessment of the functional state of the kidneys, each of the developed methods has its own drawbacks, which makes it impossible to form an integral approach to the diagnosis. The set of mutually confirming methods, including the determination of the levels of cystatin C and uric acid in the blood, the GFR calculations for cystatin C, the identification of the degree of albuminuria and ultrasound of the kidneys, applied in our study with the patients with AH and the patients with AH along with type 2 DM provides an opportunity to assess the preclinical kidney dysfunction and along with the standard methods to more clearly determine the degree of damage to the renal parenchyma.

CONCLUSIONS

1. In the preclinical stage of the development of renal dysfunction in the patients with AH and the patients with AH along with type 2 DM (the GFR in the range of 90-60 ml/min/1.73 m^2) there is no correspondence between the creatinine level and a decrease in the GFR, therefore, the assessment of the functional state of the kidneys based on the calculation of the plasma creatinine level does not allow to significantly determine the degree of their damage [9].
2. At the initial stage of renal dysfunction in the patients with AH and the patients with AH along with type 2 DM, a statistically significant increase in the concentration of cystatin C in the blood ($p < 0.001$) and a decrease in the GFR calculated from it are revealed. The high information content of the GFR calculations for cystatin C in comparison with the GFR calculations for creatinine is due to the lack of dependence on age, gender, and muscle mass [5].
3. An increase of cystatin C concentration in the the blood serum indicates preclinical kidney damage in the patients with AH and the patients with AH along with type 2 DM in 20.6% and 36% of cases, respectively. If it is possible to reveal early renal dysfunction in 27% of cases using integrated methods of GFR calculations for creatinine and determining the degree of albuminuria, then the study of cystatin C increases these chances to 52%, which indicates a higher diagnostic sensitivity of the latter one [6].
4. A significant increase in the volume of the kidneys of the patients with AH and the patients with AH along with type 2 DM (224.6 ± 3.17 cm^3; $p < 0.001$) is diagnosed much earlier than the appearance of clinical signs of kidney damage. In the patients with AH of I-II degrees and the patients with AH along with type 2 DM, there is a decrease in the linear velocity of renal blood flow and an increase in the indices of intrarenal vascular resistance [1,3, 7].

5. Determination of the levels of cystatin C, uric acid in the blood, the degree of albuminuria, the calculation of GFR for cystatin, the study of biometric parameters of the kidneys and renal blood flow in combination can increase the efficiency of early detection of renal dysfunction [4, 8].

PRACTICAL RECOMMENDATIONS

1. It is necessary to investigate the content of cystatin C in blood and use the formulas for GFR calculations based on it in order to study the manifestation of preclinical renal dysfunction.
2. It is necessary to check the renal excretion of albumin as a marker of probable kidney damage in patients with AH and patients with AH along with type 2 DM.
3. For an objective characterization of preclinical kidney damage in patients with AH and patients with AH along with type 2 DM, it is recommended to carry out the proposed studies, including sonography methods in conjunction with laboratory tests.

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LIST OF ABBREVIATIONS

AH - arterial hypertension
BMI - body mass index
BP - blood pressure
CrCl - creatinine clearance
CKD - Chronic Kidney Disease
CVD - cardiovascular disease
DM - diabetes mellitus
DN - Diabetic Nephropathy
GFR - glomerular filtration rate
HF - heart failure
PKD - Preclinical Kidney Disease
TC - total cholesterol

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