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## THE CONTENT OF NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN IN THE BLOOD SERUM OF YOUNG PATIENTS WITH ARTERIAL HYPERTENSION

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The purpose of the work was to study the content of neutrophil gelatinase associated lipocalin in blood serum and its relationship with parameters of intrarenal hemodynamics in young patients with arterial hypertension. It was established that in such patients the maximum value of neutrophil gelatinase associated lipocalin content in blood serum was characteristic of individuals with a reduced glomerular filtration speed compared to patients with preserved glomerular filtration speed and healthy individuals. The duplex scan data of the renal arteries showed that in patients with arterial hypertension of young age a decrease in the end-diastolic velocity of arterial blood flow and increasing the resistance index was recorded, more pronounced when the rate of glomerular filtration decreases, that confirms their diagnostic significance in the assessment of morpho-functional changes in the kidneys.

**Key words:** neutrophil gelatinase associated lipocalin, arterial hypertension in young people, duplex scanning of the renal arteries, glomerular filtration speed.

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## ВМІСТ ЛІПОКАЛІНУ, АСОЦІЙОВАНОГО З ЖЕЛАТИНАЗОЮ НЕЙТРОФІЛІВ, У СИРОВАТЦІ КРОВІ У ХВОРИХ НА АРТЕРІАЛЬНУ ГІПЕРТЕНЗІЮ МОЛОДОГО ВІКУ

Метою роботи було вивчення вмісту ліпокаліну, асоційованого з желатиназою нейтрофілів у сироватці крові та його взаємозв'язку з параметрами внутрішньониркової гемодинаміки у хворих на артеріальну гіпертензію молодого віку. Встановлено, що у пацієнтів з артеріальною гіпертензією молодого віку максимальне значення вмісту ліпокаліну, асоційованого з желатиназою нейтрофілів у сироватці крові визначено в осіб зі зниженою швидкістю клубочкової фільтрації в порівнянні з пацієнтами зі збереженою швидкістю клубочкової фільтрації та здоровими особами. За даними дуплексного сканування ниркових артерій встановлено, що у пацієнтів з артеріальною гіпертензією молодого віку реєструвалось зниження кінцевої діастолічної швидкості артеріального кровотоку і підвищення індексу резистентності, більш виражені при зниженні швидкості клубочкової фільтрації, що підтверджує їх діагностичну значущість при оцінці морфо-функціональних змін нирок.

**Ключові слова:** ліпокалін, асоційований з желатиназою нейтрофілів, дуплексне сканування ниркових артерій, артеріальна гіпертензія у молодих, швидкість клубочкової фільтрації

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Arterial hypertension (AH) prevalence among young people aged 18 to 39 years has been of 3.7 % to 8.6 % of all patients [10]. According to research by other authors, the prevalence of AH in the age group of 20–40 years is 17.9 %, which may lead to an increased burden of cardiovascular disease in the population [9]. The prognosis for AH is defined as the degree of increase in blood pressure (BP), as well as the severity of structural and functional changes on the part of target organs: heart, brain, kidneys and blood vessels. It has been established that the presence of target organ lesions in AH increases the risk of cardiovascular complications at any level of BP [6]. Therefore, the search for biomarkers that reflect early pathological changes in target organs, namely the heart and kidneys, is an urgent issue in modern medicine. First of all, this applies to manifestations of AH at a young age, when the morphological changes of the heart and kidneys are reversible, and therapeutic interventions are most effective.

One of the representatives of a large group of lipocalins is neutrophil gelatinase-associated lipocalin (NGAL). NGAL, kidney injury molecule-1 (KIM-1), etc. refer to molecules, which are expressed by tubular epitheliocytes and reflect the degree of tubulointerstitial damage and fibrosis [2].

It has been established that the leading method of studying intrarenal blood flow in kidney pathology is ultrasound using Doppler techniques [5]. Therefore, studying the state of intrarenal hemodynamics in the early stages of kidney damage with hypertension in young people is relevant.

**The purpose** of the study was to establish the content of lipocalins is neutrophil gelatinase-associated lipocalin in blood serum and its relationship with parameters of intrarenal hemodynamics in young patients with arterial hypertension.

**Materials and methods.** 64 patients aged 25–39 years (mean age  $30.4 \pm 2.5$  years) with 1st and 2nd degree AH were examined, of them, 54 men (84.4 %) and 10 women (15.6 %). Patients did not receive regular drug therapy; the duration of AH averaged  $3.1 \pm 1.7$  years. All patients were examined in accordance with the recommendations of the European Society of Hypertension and the European Society of Cardiology (ESH/ESC, 2018).

All subjects who were examined signed an informed consent to participate in the study.

Exclusion criteria were: secondary forms of hypertension, professional athletes, regular use of antihypertensive drugs in the anamnesis, heart rhythm disorders, chronic kidney diseases, liver dysfunction and endocrine pathology.

All examined persons underwent a general clinical examination, which included a survey to identify risk factors for cardiovascular diseases, doctor's inspection, anthropometric measurements, physical examination, measurement of office BP, heart rate (HR), general blood and urine analysis (with determination of microalbuminuria), biochemical blood analysis with determination of fasting glucose, lipid metabolism parameters, creatinine (with calculation of glomerular filtration rate (GFR) according to the CKD-EPI formula (Chronic Kidney Disease Epidemiology Collaboration) [1]. Chronic kidney disease (CKD) is defined according to Clinical Practice Guideline for Evaluation and Management Chronic Kidney Disease (KGIGO 2012). NGAL content was determined in the blood serum of all examinees using commercial kits for enzyme immunoassay from the company “Hycult Biotech” (Netherlands).

$25 \text{ kg/m}^2 \leq \text{BMI} < 30 \text{ kg/m}^2$  was considered the criterion of excess weight, the obesity criterion is  $\text{BMI} \geq 30 \text{ kg/m}^2$ . The criteria for abdominal obesity were waist circumference (WC) in women  $\geq 80$  cm, in men  $\geq 94$  cm; values of WC/HC –  $\geq 0.94$  in men and  $\geq 0.80$  in women.

The blood pressure daily monitoring (BPDm) was performed using the “ABPM-02” device (Meditech, Hungary).

Duplex scanning of the renal arteries was carried out in the area of the distal parts of the main trunk of the renal artery on an ultrasound scanner “GE Medical Systems” (Germany) with a convex sensor with a frequency of 2.25–3 MHz and a sensor with a frequency of 1–7 MHz in the modes of color Doppler mapping and pulsed wave to determine the state of renal hemodynamics, peak systolic (Vps, cm/s), end-diastolic arterial blood flow velocities (Ved, cm/s) were assessed. The resistance index was determined by the formula  $\text{RI} = (\text{Vps} - \text{Ved}) / \text{Vps}$ .

According to the CKD-EPI formula, patients were divided into 2 groups. The 1st group included patients with preserved GFR –  $\text{rGFR} > 90 \text{ ml/min/m}^2$  – 48 (75 %) people, and the 2nd – with stage 2 chronic kidney disease (CKD) with  $\text{rGFR} 60\text{--}89 \text{ ml/min/m}^2$  – 16 (25 %) patients. Patients with  $\text{GFR} \leq 59 \text{ ml/min/m}^2$  were not included in the study.

The control group consisted of 15 normotensive individuals aged 25–39 years (average age  $30.2 \pm 3.1$  years) with no obvious signs of kidney, cardiovascular or other diseases (Table 1).

Table 1

**Clinical characteristics of patients depending on the presence of CKD**

Parameters	Group 1 (n=48)	Group 2 (n=16)	Control (n=15)
Mean age, years	$27.5 \pm 2.4$	$35.3 \pm 3.6^*$	$30.2 \pm 3.1$
Men / women	41/7	13/3	13/2
Duration of hypertension, years	$3.0 \pm 1.1$	$3.2 \pm 1.5$	-
Smoking, n (%)	17 (35.4 %)	7 (43.7 %)	-
Family history of hypertension, n (%)	28 (58.3 %)	9 (56.3 %)	-
Family history of early cardiovascular events, n (%)	16 (33.3 %)	5 (31.2 %)	-
BMI, $\text{kg/m}^2$ .	$24.7 \pm 0.5$	$28.6 \pm 1.6^*$	$24.8 \pm 0.8$
Abdominal obesity, n (%)	3 (6.2 %)	6 (37.5 %)	-
Dyslipidemia, n (%)	7 (14.6 %)	8 (50.0 %)	-

Notes: \* –  $p < 0.05$  between groups 1 and 2.

It was established that the patients of group 1 were significantly younger in comparison with patients of the 2nd group. There were no significant differences in the average duration of hypertension

between groups of patients. 24 patients (37.5 %) among the examined patients smoked. The percentage of smokers in the selected groups did not differ significantly.

During the analysis of metabolic factors, it was established that the total BMI of the examined patients was  $26.7 \pm 0.9$  kg/m<sup>2</sup>. It was noted that BMI in group 1 (without CKD) was significantly lower than in group 2 patients ( $p < 0.05$ ).

Among all the examined patients, an increase in the level of total cholesterol over 5.0 mmol/l was noted in 15 patients (23.4 %), an increase in the level of triglycerides  $\geq 1.7$  mmol/l in 9 patients (14.1 %) and a decrease in the level of high-density lipoproteins in men  $\leq 1.0$ , in women  $\leq 1.2$  mmol/l was detected in 5 (7.8 %) patients. The frequency of detection of dyslipidemia at the level of CKD  $\geq 5.0$  mmol/l was the highest in patients of group 2 – 50.0 %. In the same group, there was also the maximum frequency of detection of TG level  $\geq 1.7$  mmol/l (37.5 %). The frequency with a decrease in the level of HDL was higher in group 2 – 31.2 %.

The obtained results were processed by the methods of variational statistics using the computer program Statistica 10.0. The data were presented in the generally accepted form ( $M \pm m$ ), where  $M$  is the arithmetic mean, and  $m$  is the error of the arithmetic mean. The results were considered statistically significant with a margin of error of less than five percent ( $p < 0.05$ ). The Student's t-test was used to analyze the significance of the differences between the two groups in terms of the severity of the indicator, which is measured by a number. To assess the degree of connectedness or synchronicity in changes in indicators, the r-coefficient of linear correlation was calculated – the product of moments according to Pearson.

**Results of the study and their discussion.** It was established that there were no significant differences in the mean values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) according to clinical measurements between the groups of examined patients. No significant differences in heart rate (HR) were found between the groups.

The average daily indicators of SBP and DBP did not differ between the groups of examined patients, which was explained by the criteria for inclusion in the study of patients with hypertension of the 1st and 2nd degree. The results of the BPDM analysis did not allow determining the influence of these indicators on the presence and stage of CKD.

The functional state of the kidneys as a target organ for hypertension in young patients was considered. To assess kidney function in patients with hypertension, microalbuminuria (MAU), serum creatinine, and glomerular filtration rate (GFR) were evaluated.

The average rGFR in patients of group 1 was  $100.5$  [ $90.3$ – $106.9$ ] ml/min/m<sup>2</sup> and  $83.8$  [ $78.5$ – $89.8$ ] ml/min/m<sup>2</sup> in patients of group 2.

Fig. 1 shows the content of NGAL in the blood serum of the examined persons.

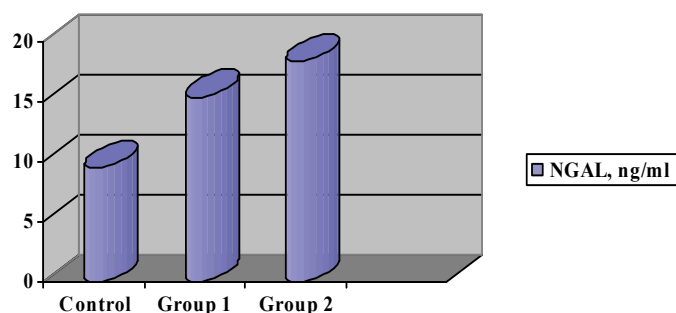


Fig. 1. NGAL content (ng/ml) in the blood serum of patients with hypertension with preserved GFR (group 1), patients with hypertension and reduced GFR (group 2), and the control group.

The results of duplex scanning of renal arteries in examined patients and healthy individuals are presented in Fig. 2.

The analysis of the parameters of the duplex scan of the renal arteries showed that the peak systolic velocity of the arterial blood flow ( $V_{ps}$ ) in patients of both groups increased significantly ( $p < 0.05$ ) compared to the control group, but no significant differences were found between the experimental groups of patients.

A decrease in the end-diastolic velocity of arterial blood flow ( $V_{ed}$ ) was determined in patients of both groups ( $p < 0.001$ ) in comparison with the control group. Moreover, a more pronounced decrease in  $V_{ed}$  was noted in patients with hypertension with reduced GFR compared to the group of patients with preserved hypertension GFR ( $p < 0.05$ ).

The maximum values of NGAL content in blood serum were determined in the group with reduced GFR –  $18.34$  ( $16.18$ – $20.17$ ) ng/ml, which was 1.9 times ( $p < 0.001$ ) higher than the value of the control group and 1.2 times ( $p < 0.05$ ) patients of group 1. In patients with hypertension and preserved GFR (group 1), the content of NGAL in blood serum was  $15.37$  ( $12.82$ – $17.15$ ) ng/ml, which was 1.6 times ( $p < 0.001$ ) higher than the value of the group of healthy individuals.

A significant increase in the resistance index (RI) was established in patients of both groups compared to the control group ( $p < 0.001$ ). At the same time, RI significantly increased in the group of patients of group 2 in comparison with group 1 ( $p < 0.05$ ).

Therefore, when performing a duplex scan of the renal arteries in patients with hypertension with reduced GFR, a decrease in Ved ( $p < 0.001$ ) and an increase in RI ( $p < 0.001$ ) can be used as markers of early kidney dysfunction.

When conducting a correlation analysis in patients with hypertension with reduced GFR, it was noted that the content of NGAL was directly correlated with the concentration of creatinine in blood serum ( $r = 0.39$ ;  $p < 0.05$ ). Direct correlations of the resistance index (RI) with the content of NGAL ( $r = 0.42$ ;  $p < 0.05$ ) and creatinine concentration in blood serum ( $r = 0.38$ ;  $p < 0.05$ ), as well as between Ved and CKD-EPI GFR ( $r = 0.33$ ;  $p < 0.05$ ) were established. Inverse correlations were found between Ved and NGAL content in blood serum ( $r = -0.43$ ;  $p < 0.05$ ), as well as between RI and CKD-EPI GFR ( $r = -0.32$ ;  $p < 0.05$ ).

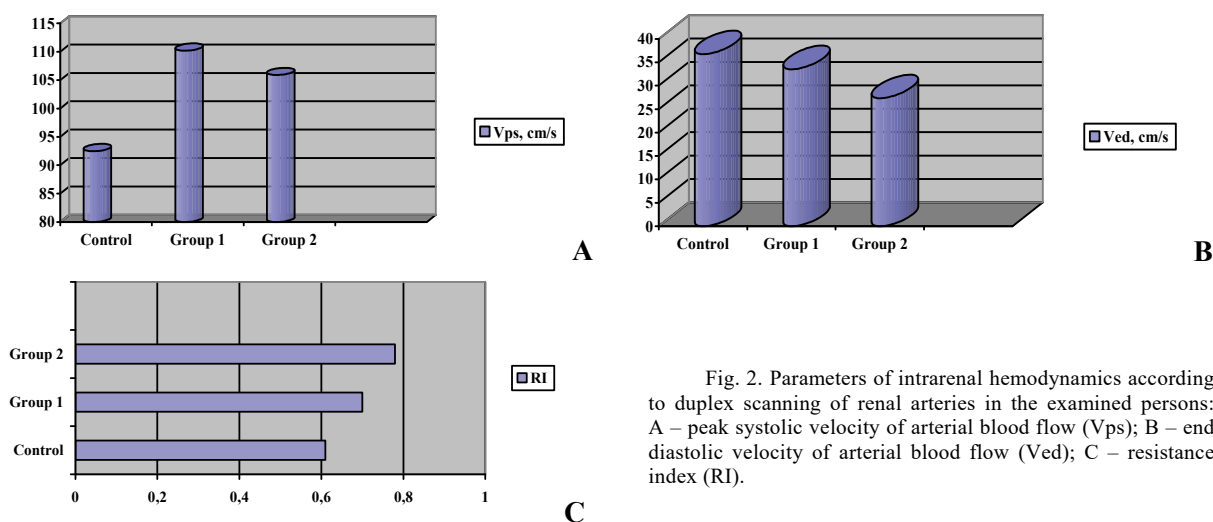


Fig. 2. Parameters of intrarenal hemodynamics according to duplex scanning of renal arteries in the examined persons: A – peak systolic velocity of arterial blood flow (Vps); B – end diastolic velocity of arterial blood flow (Ved); C – resistance index (RI).

Arterial hypertension is a common cause of CKD, and therefore, patients with high blood pressure have an increased risk of renal dysfunction and the development of CKD [3], which makes it urgent to search for early markers of kidney damage in patients with hypertension in order to slow down the progression of CKD. Moreover, there are still not enough studies devoted to the determination of early signs of kidney damage in young Ukrainians with hypertension.

In our study, GFR was preserved in most patients, which may be due to the young age of the patients and the short history of hypertension.

It was established that a decrease in the level of GFR to  $75 \text{ ml/min/m}^2$  is unfavorable in terms of cardiovascular prognosis [7]. According to our data, GFR below  $75 \text{ ml/min/m}^2$  was determined in 18.7 % of patients of group 2.

From today's point of view, MAU is considered as a criterion for initial kidney dysfunction and generalized damage to the microvascular bed [12]. We determined MAU in 37.5 % of patients of group 2, and in obese patients.

It has been shown that patients with hypertension have an increase in the content of NGAL in urine, which indicates impaired kidney function, regardless of the severity of hypertension and its duration [2]. This is evidenced by the data obtained by us: an increase in the level of NGAL in blood serum was found in patients with hypertension at a young age, with a maximum value when GFR is reduced. Therefore, an increase in the content of NGAL in blood serum was detected at an optimal level of MAU and a preserved GFR.

Higher levels of circulating NGAL have previously been shown to correlate with high blood pressure and insulin resistance, based on the role of NGAL in oxidative stress, endothelial dysfunction, inflammation, and hypertension [11]. The results of our study may indicate that glomerular and tubular kidney damage is observed in hypertension, which allows us to consider the NGAL biomarker as an early marker of nephron damage in hypertension.

The results of a duplex scan of the renal arteries indicate an increase in the peripheral resistance index – RI, which is more pronounced in patients with hypertension and reduced GFR (group 2), which indicates an increase in intraglomerular pressure and a faster progression of renal failure.

It was previously established that RI reflects disturbances in systemic hemodynamics [8]. It has been shown that an increase in RI is a risk factor for the development of cardiovascular diseases in elderly patients, and is also associated with a high risk according to the Framingham scale [4]. The data of our study made it possible to establish that the determination of RI during duplex scanning of renal arteries of various calibers makes it possible to determine the signs and severity of impaired function and structure of the kidneys: a decrease in GFR and the thickness of the renal parenchyma.

### Conclusions

1. In patients with hypertension at a young age, when studying a potential biomarker of renal dysfunction, it was established that the maximum value of NGAL content in blood serum was determined in individuals with hypertension and reduced GFR compared to healthy individuals and patients with hypertension with preserved GFR.

2. According to the duplex scan of the renal arteries, it was established that in patients with hypertension at a young age, a decrease in the end-diastolic velocity of arterial blood flow (Ved) and an increase in the resistance index (RI) were recorded, which were more pronounced when the GFR decreased, which confirms their diagnostic significance in the assessment of morpho-functional changes kidney.

### References

1. Katerenchuk IP. Klinichna otsinka, diahnostychnie i prohnostychnie znachennia deiakykh suchasnykh laboratornykh doslidzhen u patsientiv z ishemichnoyiu khvoroboiu sertsia. *Kardyolohyia: ot nauky k praktyke*. 2019;3(37):70–81. doi: <https://doi.org/10.30702/card.sp.2019.08.037/0307081> [in Ukrainian]
2. Danquah M, Owiredu WKBA, Jnr BAE, Serwaa D, Odame Anto E, Pephrah MO, Obirikorang C, Fondjo LA. Diagnostic value of neutrophil gelatinase-associated lipocalin (NGAL) as an early biomarker for detection of renal failure in hypertensives: a case-control study in a regional hospital in Ghana. *BMC Nephrol*. 2023 Apr 26;24(1):114. doi: 10.1186/s12882-023-03120-6.
3. GBD Chronic Kidney Disease Collaboration. Global, regional, and national burden of chronic kidney disease, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2020, 395, 709–733.
4. Heine GH, Gerhart MK, Ulrich C, Köhler H, Girndt M. Renal Doppler resistance indices are associated with systemic atherosclerosis in kidney transplant recipients. *Kidney Int*. 2005;68(2):878–885.
5. Lameire NH, Levin A, Kellum JA, Cheung M, Jadoul M, Winkelmayer WC, Stevens PE; Conference Participants. Harmonizing acute and chronic kidney disease definition and classification: report of a Kidney Disease: Improving Global Outcomes (KDIGO) Consensus Conference. *Kidney Int*. 2021 Sep;100(3):516–526. doi: 10.1016/j.kint.2021.06.028.
6. Meher M, Pradhan S, Pradhan SR. Risk Factors Associated With Hypertension in Young Adults: A Systematic Review. *Cureus*. 2023 Apr 12;15(4): e37467. doi: 10.7759/cureus.37467.
7. Mulè G, Castiglia A, Cusumano C, Scaduto E, Geraci G, Altieri D, Di Natale E, Cacciatore O, Cerasola G, Cottone S. Subclinical Kidney Damage in Hypertensive Patients: A Renal Window Opened on the Cardiovascular System. *Focus on Microalbuminuria. Adv Exp Med Biol*. 2017; 956:279-306. doi: 10.1007/5584\_2016\_85.
8. Ohta Y, Fujii K, Arima H, Matsumura K, Tsuchihashi T. Increased renal resistive index in atherosclerosis and diabetic nephropathy assessed by Doppler sonography. *J Hypertens*. 2005;23(10):1905–1911.
9. Sidenur B, Shankar G. A Cross-Sectional Study of Hypertension among 20-40 Years Old Residing in an Urban Area of Bagalkot City, North Karnataka. *Indian J Community Med*. 2023 Jan-Feb;48(1):98–102. doi: 10.4103/ijcm.ijcm\_255\_22.
10. Shin D, Choi J, Lee HY. Suboptimal control status of young hypertensive population. *Clin Hypertens*. 2023 May 1;29(1):13. doi: 10.1186/s40885-023-00237-6.
11. Song E, Fan P, Huang B, Deng H, Cheung BMY, Félétou M, et al. Deamidated lipocalin-2 induces endothelial dysfunction and hypertension in dietary obese mice. *J Am Heart Assoc*. 2014;3(2): e000837
12. Wang Y, Li F, Chu C, Zhang X, Zhang XY, et al. Early life body mass index trajectories and albuminuria in midlife: A 30-year prospective cohort study. *EClinicalMedicine*. 2022 Apr 28; 48:101420. doi: 10.1016/j.eclinm.2022.101420.

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