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POTENTIAL CIRCULATING BIOMARKERS OF ATHEROSCLEROTIC PLAQUE VULNERABILITY IN PATIENTS IN THE EARLY RECOVERY PERIOD OF ATHEROTHROMBOTIC STROKE

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Despite the informative value of ultrasound examination of atherosclerotic vascular lesions, predicting the plaque vulnerability remains difficult. Circulating blood biomarkers could provide additional criteria that would allow better determination of the risk of recurrent stroke. The aim of our study was to estimate the level of C-reactive protein (CRP), low-density lipoproteins (LDL) and lipoprotein-associated phospholipase A2 (Lp-PLA2) in the blood of patients in the early recovery period of atherothrombotic stroke depending on the density of atherosclerotic plaque according to duplex scanning of cerebral vessels. Clinical and laboratory analysis of 69 men and 61 women aged (60.42 ± 7.40) years in the early recovery period of atherothrombotic stroke was conducted. Depending on the structure of the atherosclerotic plaque the examinees were divided into two groups with stable (n = 80) and unstable (n = 50) atherosclerotic layers. The blood lipid spectrum was examined on a biochemical analyzer Screen master, the level of CRP was determined with a diagnostic kit and that of Lp-PLA2 by ELISA. Significantly higher levels of LDL, CRP and Lp-PLA2 were observed in patients with unstable atherosclerotic plaque compared to patients with stable atherosclerotic plaque in the early recovery period of atherothrombotic stroke. The increased level of LDL, CRP, and Lp-PLA2 in patients with cerebral atherosclerosis can be considered as an indicator of the vulnerability of atherosclerotic plaques, prone to rupture, and as a prognostic marker of repeated acute ischemic events.

K e y w o r d s: low-density lipoproteins, C-reactive protein, lipoprotein-associated phospholipase A2, atherosclerotic plaque, recovery period of ischemic stroke.

Detection of atherosclerotic lesions of cerebral vessels is traditionally based on ultrasound examination with measurement of stenosis lumen and characteristics of atherosclerotic plaque. The most frequent mechanism involved in the pathogenesis of atherothrombotic stroke is the rupture of an atherosclerotic plaque with subsequent thrombus formation and distal embolism [1-3]. Differences between plaques with the same degree of stenosis indicate that the nature of the atherosclerotic plaque is an important determinant and marker of “active” cerebral artery damage. Atherosclerotic plaques are considered “hard” when their composition is predominantly collagenous or calcified; “soft”, in the case of intraplaque hemorrhage or when they contain atheromatous fragments. Soft plaques are unstable and are often the cause of acute cardiovascular events. Solid plaques are stable and characterized by an asymptomatic course [4, 5].

However, despite the informative nature of ultrasound examination of atherosclerotic vascular lesions, predicting the vulnerability of plaques remains ambiguous and requires further investigation. Biochemical circulating blood biomarkers could characterize the activity of the atherosclerotic process, predict the risk of progression of atherosclerosis, prevent the recurrence of stroke by stabilizing the situation [3, 5]. Having processed the literature data in this direction, we decided to investigate and find out the difference in concentration changes in the blood of potential biomarkers of destabilization of atherosclerotic plaques, which could increase the prognostic value of atherothrombosis.

Traditionally, the pathophysiology of atherothrombosis is closely related to the lipid theory. It is well-known that lowering LDL-C reduces the risk of fatal or non-fatal heart attack or stroke by 30%. However, according to the results of the PROVE-IT

study, despite achieving the target LDL-C level according to the recommendations, 23% of stroke patients had recurrent cardiovascular events during the following two years [2, 3, 5].

According to the results of the MONICA, WOSCOPS study, the level of lipoprotein-associated phospholipase A2 (Lp-PLA2) should be measured to improve the prediction of CVD risk and use the obtained data in addition to traditional risk factors [6, 7].

In the biological sense, Lp-PLA2 is a pro-inflammatory free enzyme, specific for vascular inflammation, which exhibits physiological activity in the intima of the artery. Lp-PLA2 in high concentration is found in plaques prone to rupture from where it enters the bloodstream [2, 6-8]. The clinical threshold at which a patient is classified as at high risk of cerebrovascular events is Lp-PLA2 ≥200 ng/ml. This value was obtained based on a review of studies that showed a marked increase in the risk of cardiovascular events in patients exceeding this threshold [4, 7, 9]. Thus, moderate-risk individuals with Lp-PLA2 values 200 ng/ml were reclassified and acquired the status of patients at high risk of vascular events. With elevated Lp-PLA2 after a stroke, mortality within 1 year increased 5 times. It has been proven that statins reduce Lp-PLA2 independently of LDL reduction [3, 7, 10].

A number of studies have demonstrated that the activity of vascular inflammation, assessed by increasing the levels of inflammatory markers and acute-phase proteins, is accompanied by a high risk of developing atherosclerotic plaque damage [2, 8, 11]. It is believed that pro-inflammatory activation, and not the degree of atherosclerotic stenosis, is the factor that ensures the severity of subsequent ischemic brain tissue damage. According to the results of the JUPITER study, it was established that patients with high levels of C-reactive protein (CRP) had a significantly higher cardiovascular risk, even in the presence of target levels of LDL-C in the blood. Achieving a CRP level of up to 1 mg/l in the blood reduced the cardiovascular risk in these subjects by 79%. It should be noted that statins, regardless of the hypolipidemic effect, reduce the concentration of CRP in blood plasma by 20-30%, which is considered one of the goals of therapy [1, 12, 13]. Möhlenkamp et al. found in their research that if the CRP index or coronary artery calcification is added to the Framingham risk scale, they will improve the prediction of the risk of cardiovascular events in the general population.

However, despite the obvious importance of markers of inflammation in atherosclerosis, their role in the diagnosis and prevention of disease progression is practically not taken into account [2, 8, 14].

Based on the above, the issue of studying predictors of atherothrombotic complications circulating in the blood in patients with an ischemic stroke in the early recovery period is relevant and of significant scientific interest.

The purpose of the study is to study changes in C-reactive protein, low-density lipoproteins, and lipoprotein-associated phospholipase A2 in the blood of patients in the early recovery period of atherothrombotic stroke, depending on the density of atherosclerotic plaque according to the duplex scanning of vessels.

Materials and Methods

The atherothrombotic subtype of stroke was ascertained based on the results of CT scan of the brain, perfusion CT of the brain, duplex scanning of the vessels of the head and neck, ECG, coagulogram, expanded lipidogram, biochemical blood analysis during the acute period of stroke during hospitalization. The rhythm of the heart in all the examined was correct, sinus. The localization of the atherosclerotic plaque corresponded to the side of the affected cerebral hemisphere. 67 patients underwent thrombolytic therapy. All subjects were taking antiplatelet agents, statins, hypotensive and hypoglycemic drugs in the presence of diabetes for the purpose of secondary prevention of stroke.

At the revisit of 130 patients in the early recovery period of a stroke (3-6 months after the onset of acute cerebrovascular accident), repeated duplex scanning of the vessels of the head and neck was performed and the levels of CRP, LDL and Lp-PLA2. Among the examined were 69 men and 61 women aged (60.42 ± 7.40) years. The control group consisted of 30 practically healthy persons (16 men and 14 women) with no history of severe somatic pathology and disorders of cerebral circulation aged (58.7 ± 6.3) years.

Ultrasound duplex scanning of the neck vessels with the determination of the degree of atherosclerotic stenosis and the nature of the atherosclerotic plaque was performed on a Siemens Acuson X 300 device with a linear multifrequency sensor from 4–10 mHz) according to standard methods.

The blood lipid spectrum was determined using the enzymatic calorimetric method in blood

plasma on a biochemical analyzer Screen master lab manufactured by Hospitex diagnostic (Germany). The level of total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL) was determined. The content of low-density lipoproteins (LDL) was calculated according to the formula of W. Freedwald:

LDL = total cholesterol –
– (HDL cholesterol + TG/2.2).

Determination of C-reactive protein was carried out by the "CRP-latex test", according to which the measurement is carried out using a diagnostic kit of domestic production (ToV NZL "Granum", Kharkiv). The setting of the test in the study of blood serum was carried out according to the instructions.

The amount of Lp-PLA2 was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Diazyme Laboratories Inc., Poway, California, USA). Plasma was added to the microplate wells with anti-Lp-PLA2 monoclonal antibodies (2C10) and incubated for 10 min at room temperature. Then the second monoclonal antibodies (4B4), labeled with horseradish peroxidase enzyme, were added and incubated for 180 min. Wells were washed and tetramethylbenzidine substrate was added. After 20 min of incubation, absorbance at 450 nm was measured, which is directly proportional to the concentration of Lp-PLA2 in the plasma. The concentration of Lp-PLA2 is expressed in units of ng/ml.

The study was approved by the Bioethics Commission of Ivano-Frankivsk National Medical University (protocol No 21 dated 09.27.2022). Patients gave written informed consent for the above diagnostic procedures and participation in this research project.

Statistica 8 software (StatSoft, Serial STA862D175437Q) was used for statistical processing. The frequency of qualitative indicators was represented in absolute (*n*) and relative (%) frequencies. When analyzing quantitative data, it was necessary to determine the nature of the distribution of indicator values using Shpiro-Wilk's test. For quantitative data with a normal distribution, the results were represented as "M (σ)," where M is the mean value and σ is the standard deviation. For quantitative data with an abnormal distribution, "Me (Q1; Q2)" was used, where Me is the median and Q1; Q2 are quartiles. Quantitative indicators with normal distribution of values in 2 independent groups were compared using the Student's criterion. Quantitative parameters with abnormal distribution in 2 independent groups were compared using the Mann–Whitney method. A comparison of 2 independent groups for the qualitative indicator was carried out according to the exact Fisher criterion.

Results and Discussion

All examined (100%) in the early recovery period of ischemic stroke had cerebral atherosclerosis, arterial hypertension was established in 116 (89%), diabetes – in 35 (27%) patients. 36 patients (27.6%) had smoking experience of more than 10 years. The degree of stroke severity according to the NIHHS scale in the examined subjects was mild (<5 points) in 29 (22.4%), moderate (6-13 points) in 71 (54.6%) and severe (14-20 points) in 30 (23%) of patients. According to a duplex scan of the head and neck vessels, hemodynamically insignificant atherosclerotic stenoses of up to 75% were found in all patients in the early recovery period of atherothrombotic stroke. Depending on the structure of the atherosclerotic plaque, the examinees were divided into a group with stable (n = 80) and unstable (n = 50) atherosclerotic layers.

Unstable atherosclerotic plaques, which have a high degree of embologenicity and are a potential threat of recurrent ischemic stroke, mostly had a heterogeneous structure with hypoechoic inclusions, an uneven surface, and existing layers. Stable plaques were homogeneous, hyperechoic.

Comparing the levels of LDL cholesterol, their significant increase was noted in both studied groups in comparison with the control group. The highest values of LDL-C were observed in the group of patients with unstable atherosclerotic plaques, and were (3.1 (2.5:3.4)), respectively.

The increase in the level of CRP in the blood of patients with ischemic atherothrombotic stroke in comparison with the control group reached the level of reliability in both studied groups. Comparing the level of CRP in the examinees depending on the echogenicity of the atherosclerotic plaque, it was found to be higher in patients with unstable atherosclerotic plaque, which indicates the activation of the inflammatory process. According to the literature, an increase in CRP in the blood of patients can predict the development of cardiovascular diseases: low risk (<1.0 mg/l); moderate (1.0–2.9 mg/l); high (>3.0 mg/l). According to our results, patients with unstable atherosclerotic plaque have a high risk of recurrent acute vascular catastrophe.

Table. Indicators of LDL cholesterol, C-reactive protein and lipoprotein-associated phospholipase A2 in patients with atherothrombotic stroke in the early recovery period depending on the structure of the atherosclerotic plaque

Indicator (concentration)	Healthy individuals, (n = 30)	Patients with atherothrombotic stroke (n = 130)	
		With stable atherosclerotic plaques (n = 80)	With unstable atherosclerotic plaques (n = 50)
LDL cholesterol, mmol/l Me (q ; q2)	2.09 (1.9; 2.2)	2.76 (2.5; 3.2) $P < 0.05$	3.1 (2.5; 3.4) $P < 0.05$
CRP, mg/l Me (q ; q2)	0.95 (0.92; 1.1)	4.2 (3.5; 4.7) $P < 0.05$	7.46 (6.9; 7,9) $P < 0.05$, $P_1 < 0.05$
Lp-PLA2, ng/ml Me (q ; q2)	200.75 (195.1; 202.2)	250.2 (245.9; 267.3) $P < 0.05$	285.9 (265.5; 295.3) $P < 0.05$, $P_1 < 0.05$

Note: P is the reliability of the difference in patients with early reversible atherothrombotic stroke: with stable and unstable atherosclerotic plaques compared to a group of practically healthy individuals; P_1 – the reliability of the difference in indicators of the early recovery period of a stroke with unstable atherosclerotic plaques in comparison with the group of patients in the early recovery period of a stroke with stable atherosclerotic plaques; Me (q1; q2) – median and quartile

The average level of Lp-PLA2 in the blood of the subjects of the control group was 200.75 ng/ml. The concentration of Lp-PLA2 was significantly higher in patients with mild, unstable atherosclerotic plaque compared with stable. In addition, in patients with the same parameters of stenosis levels in both studied groups, significantly higher indicators were recorded in the group with mild atherosclerotic layers. That is, the increase in the concentration of Lp-PLA2 depended more on the structure of the atherosclerotic plaque than on its size. According to the literature, the interpretation of Lp-PLA2 levels in blood serum is based on the following scale: low degree of cardiovascular risk - (< 200 ng/ml); average degree of risk - (200–235 ng/ml); high degree of risk - (> 250 ng/ml). Based on our results, patients with mild atherosclerotic plaque belong to the group of high risk of stroke.

When performing a correlation analysis between the level of LDL cholesterol and Lp-PLA2 in patients in the early recovery period of atherothrombotic stroke with unstable atherosclerotic plaques, we found a strong direct correlation ($r = 0.71$, $P < 0.01$), between LDL cholesterol and CRP direct relationship of medium strength ($r = 0.56$, $P < 0.05$) and between Lp-PLA2 and CRP also direct relationship of medium strength ($r = 0.61$, $P < 0.05$).

Conclusions. Patients in the early recovery period of atherothrombotic stroke with unstable atherosclerotic plaque had significantly higher levels of LDL, C-reactive protein, and Lp-PLA2 compared with stable plaque.

Concentration increases in LDL, CRP, and Lp-PLA2 in patients with atherothrombotic stroke in combination with ultrasound characteristics of cerebral atherosclerotic plaque can be considered as highly embolic biomarkers of atherosclerotic plaque vulnerability, which will contribute to the improvement of diagnosis and prevention of recurrent stroke by optimizing treatment options.

Prospects for further research. In our opinion, it would be interesting to study the levels of LDL, C-reactive protein and lipoprotein-associated phospholipase A2 in the dynamics for a longer time in parallel with the correction of the applied therapy.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http:// ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ПОТЕНЦІЙНІ ЦИРКУЛЮЮЧІ БІОМАРКЕРИ ВРАЗЛИВОСТІ АТЕРОСКЛЕРОТИЧНОЇ БЛЯШКИ У ПАЦІЄНТІВ У РАННЬОМУ ПЕРІОДІ ВІДНОВЛЕННЯ ПІСЛЯ АТЕРОТРОМБОТИЧНОГО ІНСУЛЬТУ

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Незважаючи на інформативність УЗД атеросклеротичного ураження судин, прогнозування вразливості бляшок залишається складним. Біомаркери, що циркулють у крові можуть надати додаткові критерії, які дозволять краще визначати ризик повторного інсульту. Метою нашого дослідження було оцінити рівень С-реактивного протеїну (CRP), ліпопротеїнів низької щільності (LDL) та ліпопротеїн-асоційованої фосфоліпази А2 (Lp-PLA2) у крові пацієнтів раннього відновного періоду атеротромботичного інсульту, в залежності від щільності атеросклеротичної бляшки за даними дуплексного сканування судин головного мозку. Проведено клініко-лабораторне дослідження 69 чолоків та 61 жінки віком (60,42 ± 7,40) років у ранньому відновному періоді атеротромботичного інсульту. Залежно від структури атеросклеротичної бляшки обстежених було розподілено на дві групи зі стабільним (n = 80) і нестабільним (n = 50) атеросклеротичними бляшками. Ліпідний спектр крові досліджували на біохімічному аналізаторі Screen master, рівень CRP визначали діагностичним набором, Lp-PLA2 – методом ELISA. У пацієнтів із нестабільною атеросклеротичною бляшкою в ранньому відновному періоді атеротромботичного інсульту спостерігали достовірно вищі рівні LDL, CRP та Lp-PLA2 порівняно з пацієнтами зі стабільною атеросклеротичною бляшкою. Підвищення рівня LDL, CRP і Lp-PLA2 у хворих на церебральний атеросклероз можна розглядати як показник вразливості атеросклеротичних бляшок, схильних до розриву, і як прогностичний маркер повторних гострих ішемічних подій.

Ключові слова: ліпопротеїни низької щільності, С-реактивний протеїн, ліпопротеїн-асоційована фосфоліпаза А2, атеросклеротична бляшка, відновний період ішемічного інсульту.

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