MARKERS OF ATHEROSCLEROSIS LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 AND E-SELECTIN AND VASCULAR RISK FACTORS IN PATIENTS WITH CAROTID STENOSIS

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The aim of this study is to evaluate serum level biomarkers of atherosclerosis lipoprotein-associated phospholipase A2 and E-selectin in patients with atherosclerotic carotid stenosis with different clinical manifestation in associated with vascular risk factors.

Materials and methods: A total 106 patients with atherosclerotic carotid stenosis (74 men and 32 women, aged from 31 to 74 years, mean 62.6±0.9) were included: with acute ipsilateral atherothrombotic stroke (35), history of stroke and carotid endarterectomy (41) and 30 patients with asymptomatic carotid stenosis. The control group consist of 20 health subjects without cardiovascular disease. All participants underwent duplex sonography. Lipoprotein-associated phospholipase A2 and E-selectin was measured using commercially available (ELISA) kit. Results: The level of lipoprotein-associated phospholipase A2 was in general 35.664±3.537 ng/ml, which was significantly higher (M-W U=10, p=1.023136 ×10-11 <0.05) than in the control group (9.296±0.935 ng/ml). Level was significantly higher in groups of symptomatic patients who underwent carotid endarterectomy (p=0.04893), and proportion patients with high degree stenosis >70% was greater in this group. The level of E-selectin in the study patients was significantly higher (7.65±0.246 pg/ml) than in the control group (3.10±0.503 pg/ml) p<0.05. No association the serum level of lipoprotein-associated phospholipase A2 and E-selectin with common stroke risk factor such as hypercholesterinemia, smoking and body mass index were found, but positive correlation of lipoprotein-associated phospholipase A2 with E-selectin was significant (p=0.00085).

Conclusions: Increasing plasma level lipoprotein-associated phospholipase A2 and E-selectin in patients with the carotid atherosclerotic stenosis were observe. Statistically significant correlation between the level of lipoprotein-associated phospholipase A2 and E-selectin were found in symptomatic carotid atherosclerotic stenosis

Keywords: atherosclerotic carotid stenosis, ischaemic stroke, vascular risk factors, lipoprotein-associated phospholipase A2, E-selectin

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a close relationship between the concentration of E-selectin and carotid atherosclerosis [8; 9]. However, cell adhesion biomarkers as VCAM and selectins have shown contradictory results and at present have no clinical application. The association between thus biomarkers in carotid atherosclerosis are no research.

The aim of this study is to evaluate serum level biomarkers of atherosclerosis Lp-PLA2 and E-selectin in patients with atherosclerotic carotid stenosis with different clinical manifestation in associated with vascular risk factors.

2. Materials and Methods
The study included patients, who were hospitalized in the period from November 2018 to January 2020 at the City Clinical Hospital No. 7 of the Kharkiv City Council, Ukraine.

A total 106 patients with atherosclerotic carotid stenosis (74 men and 32 women, aged from 31 to 74 years, mean 62.6±0.9) were recruited in study. The symptomatic group (group 1, 35 patients) had presented with acute non-lacunar ischaemic stroke in ipsilateral internal carotid artery (ICA), NIHSS 3-9. Group 2 include 41 patients who suffered from ischemic stroke and underwent carotid endarterectomy (CEA) on ipsilateral side with the mean follow-up period 35 months. When determining the indications for surgical intervention guided by generally accepted recommendations. Restenosis >50% after CEA developed in 18 (43.9 %), contralateral asymptomatic carotid stenosis >50% were observed in 15 (36.6 %) of them. Group 3 (30 patients) include asymptomatic patients were defined as those who had no history acute ischemic event. Neuroimaging (KT or MRI) was perform all patients. Exclusion criteria was history of atrial fibrillation, intracranial haemorrhage, inflammatory diseases, cancer.

The control group consisted of 31–56 age- and sex-matched 20 healthy subjects without a history of cardiac disease, hypertension or diabetes and having normal findings on physical examination, Doppler ultrasound and echocardiography. The study was conducted in accordance with the requirements of the Helsinki Declaration of the World Medical Association, the Council of Europe Convention on Human Rights and Biomedical Research, Good Clinical Practice (GCP) and approved by the Bioethics Commission of Kharkiv Medical Academy of Postgraduate Education (Protocol No. 9 of 21.11.2018). Patients who participated were included in the study after signing the informed consent.

All patients underwent duplex sonography. Carotid atherosclerotic examinations were performed with a high-resolution (10.0 MHz) color Doppler ultrasound by scanner «MINDRAY DC-40». Plaque was classified as unilateral or bilateral, regular or irregular, and heterogeneous or homogeneous. The degree of ICA stenosis was determined by the European Carotid Surgery Trial (ECST) method [10].

Laboratory testing. All fasting blood samples were drawn in the morning, centrifuged to serum, stored in a refrigerator at a temperature within the range from 2°C to 8°C, and transferred to a central laboratory. The levels of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by the direct assay method. Total cholesterol (TC), triglyceride (TG), were measured by a standard enzymatic method. The level of serum Lp-PLA2 was determined using a commercially available enzyme-linked immunosorbent assay kit (ELISA) Elabscience, USA. E-selectin was measured using commercially available (ELISA) kit (Elabscience, USA).

Statistics.
Data were described using mean and standard deviations or percentage for categorical data. Differences were tested using U-test for continuous variables or the χ² test for categorical variables. Multiple group comparisons were performed by ANOVA. Regression coefficients were calculated based on Spearman product moment. Statistical significance was assumed for P<0.05.

3. Results
Demographic and lifestyle characteristics of study population are described in Table 1. It showed a trend toward a higher prevalence of current smoking, high total cholesterol level and diabetes mellitus in symptomatic patients groups – with acute stroke and history of stroke and CEA. Any major differences from body mass index, low-density cholesterol level and myocardial infarction history was found between groups. However, in asymptomatic patients (group 3) there were significantly higher proportion subjects who used statins, antihypertensive and antiplatelet drugs.

As show in Table 1 proportion of patients with high degree stenosis > 70 % was more in group 2 after CEA. In asymptomatic patients (group 3) there was prevalence of moderate stenosis 50–69 %.

There was a statistically significant relationship between the degree of stenosis and the patient’s gender in all patients (χ²=10.129, p=0.01750, <0.05). At the same time, the proportion of women with stenosis from 50 % to 69 % significantly exceeds the proportion of men with a similar degree of stenosis (Z=2.748, p=0.00300, <0.05), and the proportion of men with 70–99 % stenosis is significantly higher than the same proportion of women (Z=2.581, p=0.00492 <0.05). Occlusion occurred in the same percentage of cases in patients of both sexes (Z=1.021, p=0.15366> 0.05). But, in asymptomatic stenosis, the number of cases with stenosis of 50–69 % and stenosis of 70–99 % was evenly distributed between men and women (χ²=1.118, p=0.29035> 0.05). In men, stenosis was observed at 62.5 [56.5; 79.0] %, and for women - 60.5 [59.0; 65.0] %, which does not constitute a statistically significant difference (M-W U=96.0, p=0.63150> 0.05).

A significant relationship between the degree of stenosis was found with smoking in the group 2. In this group, smokers had significantly more stenosis than non-smokers (M-W U=53.0, p=0.02537, <0.05).

The data of our study confirm the relationship between an increases in serum level Lp-PLA2 with the risk of adverse cerebrovascular events. Indeed, in patients, the level of Lp-PLA2 was in general (“on average”) 46.02 [29.51; 79.71] ng / ml, which was significantly higher (M-W U=10, p=1.023136>10-11, <0.05) than in the control group, in which Lp-PLA2 was observed at the level of 9.92 [4.58; 12.48] ng / ml (Table 2). Lp-
PLA2 concentration was significantly higher in group of symptomatic patients who underwent CEA. Lp-PLA 2 did not differ in men and women (M-W U=495.0, p=0.06387, >0.05).

The smoking factor did not affect the level of E-selectin (M-W U=666.0, p=0.24056, >0.05). An increased body mass index also did not have a significant effect on the level of E-selectin (M-W U=674.0, p=0.09702). The smoking factor did not affect the level of E-selectin (M-W U=495.0, p=0.09702). The smoking factor did not affect the level of E-selectin (M-W U=495.0, p=0.09702).

| Variable                           | Total n=106 | Group 1 n=35 | Group 2 n=41 | Group 3 n=30 |
|-----------------------------------|-------------|--------------|--------------|--------------|
| Age, year                         | 62±9.9      | 61±1.5       | 59±1.3       | 67±1.8       |
| Gender, male/female               | 74/32       | 29/6         | 33/8         | 12/18        |
| Arterial Hypertension*            | 89 (84.0 %) | 31 / (89.0 %) | 30 / (73.2 %) | 28/93.3 %    |
| Current smokers*                  | 64 / (60.4 %) | 30 / (85.7 %) | 22 / (53.6 %) | 12 / (40.0 %) |
| Total cholesterol ≥5 mmol/l       | 63 / (67.7 %) | 23 / (65.7 %) | 34 / (82.9 %) | 17 / (56.6 %) |
| Low density cholesterol ≥2 mmol/l | 87 / (93.5 %) | 33 / (94.3 %) | 35 / (85.4 %) | 30 / (100.0 %) |
| Body mass index ≥25               | 70 / (66.0 %) | 25 / (71.4 %) | 25 / (61.0 %) | 20 / (66.7 %) |
| Diabetes mellitus                 | 7 / (6.6 %)  | 4 / (11.4 %)  | 2 / (4.9 %)  | 1 / (3.3 %)  |
| Myocardial infarction history     | 10 / (9.4 %) | 3 / (8.6 %)   | 3 / (7.3 %)  | 4 / (13.3 %) |

Drug use
- antihypertensive*               | 71 / (67.0 %) | 24 / (68.6 %) | 21 / (51.2 %) | 26 / (86.7 %) |
- antiplatelet*                   | 65 / (61.3 %) | 16 / (45.7 %) | 24 / (58.5 %) | 25 / (83.3 %) |
- statins                         | 29 / (27.4 %) | 6 / (17.1 %)  | 10 / (24.4 %) | 13 / (43.3 %) |

Degree of ipsilateral carotid artery stenosis
- 50–69 %                         | 49 / (47.2 %) | 20 / (57.1 %) | 6 / (17.1 %)  | 23 / (76.7 %) |
- 70–99 %                         | 41 / (38.7 %) | 8 / (22.9 %)  | 26 / (63.4 %) | 7 / (23.3 %)  |
- occlusion                       | 15 / (14.2 %) | 7 / (20.0 %)  | 8 / (19.5 %)  | 0 / (0.0 %)   |

Note: * – p<0.05 the significance of the differences between patients groups 2 and 3

### Table 1

#### Table 2

| Group         | LP-PLA2 ng/ml Me [LQ; UQ] | LP-PLA2 ng/ml M ± m |
|---------------|--------------------------|---------------------|
| Control       | 9.92 [4.58; 12.48]       | 9.29±0.935          |
| Total         | 46.02 [29.51; 79.71]     | 55.66±4.537*        |
| Group 1       | 31.47 [25.20; 51.60]     | 46.41±6.421*        |
| Group 2       | 63.55 [39.69; 88.41]     | 67.64±5.742*        |
| Group 3       | 42.45 [31.20; 75.57]     | 50.53±5.560*        |

Note: * – p<0.05 the significance of the differences between patients groups and control. Me is the median; LQ, lower quartile; UQ, upper quartile. M – average; m is the standard error of the mean.

The data of our study did not show a significant relationship between the increase in the level of LP-PLA 2 and risk factors – smoking (M-W U=27310, >0.05), body mass index (R=0.006742, p=0.95267). There were also no significant differences in the level of LP-PLA 2 between patients with high (from 25) and low (less than 25) body mass index (M-W U=693, p=0.82287, >0.05). Lp-PLA 2 was not associated with total cholesterol level (R-0.040917, p=0.73822) and low-density cholesterol (R-0.003490, p=0.97729).

The level of E-selectin in the study patients was significantly higher than in the control group, however, the differences between the three study groups were insignificant (KW H (2, 80)=0.7979, p=0.6710> 0.05) (Table 3).

### Table 3

#### Table 3

| Group         | E-selectin pg/ml Me [LQ; UQ] | E-selectin pg/ml M ± m |
|---------------|-----------------------------|-----------------------|
| Control       | 3.345 [1.12; 4.745]         | 3.101±0.503           |
| Total         | 7.92 [6.465; 8.975]         | 7.65±0.246*           |
| Group 1       | 8.05 [7.12; 9.18]           | 8.07±0.430*           |
| Group 2       | 7.64 [6.20; 8.91]           | 7.49±0.408*           |
| Group 3       | 7.94 [5.49; 8.71]           | 7.42±0.446*           |

Note: * – p<0.05 the significance of the differences between patients groups and control. Me is the median; LQ, lower quartile; UQ, upper quartile. M – average; m is the standard error of the mean.

No relationship was found between the level of E-selectin and the degree of stenosis (R=-0.186836, p=0.09702). The smoking factor did not affect the level of E-selectin (M-W U=666.0, p=0.24056, >0.05). An increased body mass index also did not have a significant effect on the level of E-selectin (M-W U=674.0, p=0.09702). The smoking factor did not affect the level of E-selectin (M-W U=674.0, p=0.09702). The smoking factor did not affect the level of E-selectin (M-W U=674.0, p=0.09702).
p=0.67654, >0.05). E-selectin level was not associated with total cholesterol level (R=0.040917, p=0.68496) and low-density cholesterol (R=0.071801, p=0.55768). The correlation of Lp-PLA 2 with E-selectin, assessed for total patients, was significant, (R=0.365664, p=0.00035). In the acute stroke patients (group 1) we observed positive correlation, indicating a consistent increase in E-selectin and Lp-PLA 2 (R=0.437692, p=0.02865) (Fig. 1).

In the 2nd group of patients, the correlation of E-selectin with Lp-PLA 2 was also statistically significant (R=0.429143, p=0.01796) (Fig. 2). In the group of asymptomatic stenosis this correlation was absent (R=0.303846, p=0.13977).

Fig. 1. The relationship between level LP-PLA 2 and E-selectin in patients with atherosclerotic carotid stenosis and ipsilateral acute stroke

Fig. 2. The relationship between level LP-PLA 2 and E-selectin in patients with atherosclerotic carotid stenosis and history of stroke and CEA.

4. Discussion
An increased concentration of a biomarker or a set of biomarkers should indicate the occurrence or recurrence of ischemic stroke in asymptomatic and symptomatic patients, respectively [11]. Lp-PLA2 is also known as platelet-activating factor acetylhydrolase, an enzyme synthesized in macrophages and activating platelets that are transported in a binding state with circulating low-density lipoprotein and is abundantly expressed on atherosclerotic plaque [12]. Lp-PLA2 hydrolyzes oxidized low-density lipoprotein to form lysophosphatidylcholine, which increases monocyte adhesion, enhances the inflammatory response, and impairs endothelial function. The level of Lp-PLA2 is increase in atherosclerotic
plaques; in addition, it is intensely expressing in macrophages located in the fibrous capsule at the site of rupture [13]. A recent meta-analyse showed that elevated baseline Lp-PLA2 levels, detected by either activity or mass, are associated with increased stroke risk [14]. An increased level of circulating Lp-PLA2 was found in patients with high-grade carotid stenosis and unstable plaques who underwent carotid endarterectomy [15]. In previous study was showed that Lp-PLA2 expression was significantly higher in plaques of symptomatic patients than asymptomatic patients [16].

In our study we demonstrated expression of such markers of plaque bioactivity as Lp-PLA2 and E-selectin in symptomatic and asymptomatic patients with carotid artery stenosis. As in the study Ch. Wang et al. did not find significant association between Lp-PLA2 level and risk factors (gender, smoking, body mass index, total and low-density cholesterol level) [17]. In study S. Sakurai et al. demonstrated that serum levels of sE-selectin could be biomarkers for atherosclerosis in general populations and correlated with carotid intima-media thickness and heterogeneous plaque [18]. In our study statistically significant correlation between the expression of Lp-PLA2 and E-selectin were found in symptomatic carotid atherosclerotic stenosis. Each of these markers alone could contribute to plaque instability, and together they may create a cycle enhancing plaque instability with plaque macrophages as the center point. Along these lines, Lp-PLA2 was correlate with each of the other marker, suggesting a unique role for Lp-PLA2 in differential plaque instability.

Study limitations. Our study had several limitations. First, this study had a relative small sample. Second, estimation of stroke occurrence needs long duration of follow-up.

Prospects for further research. Further research using large samples and general population need to be done to clarify the exact role of Lp-PLA2 and E-selectin on carotid atherosclerosis and usefulness of a specific set of biomarkers and their combination with other risks markers.

5. Conclusions
The results of study showed elevated plasma level Lp-PLA2 and E-selectin in patients with the carotid atherosclerotic stenosis. This suggest that Lp-PLA2 and E-selectin may be markers of carotid atherosclerosis and have a significant impact on the risk of stroke occurrence.

Elevated the serum level Lp-PLA2 and E-selectin in patients with atherothrombotic stroke after CEA, most of them had a restenosis due to progression of atherosclerosis, comparable with asymptomatic patients reflect a carotid plaques vulnerability and play a predictive role for stroke recurrence.

The association of Lp-PLA2 and E-selectin and vascular risk factors such as hypercholesterolemia, smoking, body mass index were not found, but positive correlation of Lp-PLA2 and E-selectin were significant.

Conflict of interests
The authors declared there is no conflict of interests.

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