УДК 577.112.7:612.115

T. Katrii, Ph. D. stud., N. Shabanova, master, S. Ostapchuk, stud., O. Savchuk, D. Sci.,
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

THE CHARACTERIZATION OF THE SOLUBLE FIBRIN MONOMER COMPLEXES IN PATIENTS WITH ACUTE AND ONE YEAR POST ACUTE ISCHEMIC STROKE

It was shown that atherothrombotic and cardioembolic subtypes of ischemic stroke in acute phase of the disease accompanied with the appearance of the high concentrated soluble fibrin monomer complexes in blood plasma. But the concentration returned to the norm one year post ischemic stroke attack. Instead the concentration, the qualitative content of the year post stroke SFMC fraction was characterized by the higher diversity in comparison with acute fraction both subtypes of ischemic stroke as well as the healthy donors.

The different qualitative content of the SFMC fraction was observed for the both tested subtypes of ischemic stroke. The higher diversity of SFMC fractions was showed for the cardioembolic subtypes of ischemic stroke.

Key words: soluble fibrin monomer complexes, ischemic stroke, hemostasis.

Introduction. Stroke is a cerebrovascular accident, which is characterized by structural and morphological changes in the brain tissue, and persistent neurological deficits. This pathology is the third leading cause of death and long term disability in developed countries [1]. Although the incidence of the disease, mankind has only a superficial understanding of the processes of ischemia and changes in hemostasis that accompany it.

One of the most common cases of ischemic stroke is atherothrombotic and cardioembolic subtype. Atherothrombotic stroke mainly occurs on the background of atherosclerosis of cerebral arteries. Atherosclerotic plaque narrows the lumen and promotes thrombosis. At the heart of cardioembolic stroke, cerebral vessel occlusion is a fragment of a blood clot or other emboli, which were formed in the cavities of the heart or its valves [2, 3].

Whereas the treatment of stroke is limited, the best way to reduce the negative effects of this pathological condition is timely prevention, treatment and rehabilitation individual approach [4]. Today remains a topical study of the causes and mechanisms of stroke and its subtypes, as well as search for new biomarkers that can be used in diagnostics to identify individuals at high risk for stroke, as well as to optimize and control treatment.

One of the markers of haemostatic deviation is concentration of soluble fibrin monomer complex (SFMC), which are intermediate products of the transformation of fibrinogen into fibrin. Accumulation SFMC indicates abnormal activation of blood coagulation and in violation of dynamic balance between the functioning of blood coagulation and fibrinolysis [5]. The composition may include a SFMC native fibrinogen and its decay products. SFMC are well-known early markers of thrombophilia. Assay complex forms fibrin monomer can detect pathological process in the early stages of pre-clinical [6, 7]. Level of SFMC is not directly dependent on heparin therapy or thrombolytic agents, which allows the figure for accurate diagnosis of thrombosis.

The purpose of this study was to analyse the content of soluble fibrin monomer complex in blood plasma in patients with atherothrombotic and cardioembolic ischemic stroke in acute phase and one year after disease.

Methods. It was performed clinical and laboratory examination of 122 patients with acute ischemic stroke for the study. Depending on the subtype of stroke patients were randomized to open by two groups: group 1 – patients with atherothrombotic ischemic stroke (n = 66) and group 2 – patients with cardioembolic ischemic stroke (n = 56). One year past acute phase of disease groups consisted of 57 patients for AIS and 56 patients for CIS. The age of patients at the time of the inspection averaged 73 ± 8 years. Patients were hospitalized in I and II neurological department of the Kyiv city clinical hospital №4. The diagnosis of ischemic stroke was confirmed by neuroimaging (CT or MRI of the brain). All patients or their relatives have been warned of a clinical study and gave written consent to participate in it. On the first day of admission to the hospital all the patients received aspirin 325 mg orally, followed by a daily intake of 100 mg of aspirin internally. Since the second day patients received prophylactic dose of low molecular weight heparin. Plasma samples from 35 healthy donors were taken as a control.

Blood samples were collected from the cubital vein of all patients from 8 to 9 o'clock in the morning on an empty stomach in a plastic tube with sodium citrate (38 g/l) in the final 9:1 and stirred gently (not shaking). The mixture was centrifuged for 15 min at 2,000 g at the room temperature. The resulting plasma was frozen at -20°C. Plasma samples were defrosted by warming in a water bath (37°C) no longer than 15 min, then stirred by turning the tube and immediately moved on the ice. With this method of defrosting decreased activity of coagulation factors not occurred.

To determine the content of soluble fibrin monomer complex o-phenanthroline test was used. This method unlike the widely used ethanol and protamine sulfate test is more informative, standardized and has great diagnostic value [8, 9]. This technique is based on an assessment of the advent of plasma fibrin particles investigated after adding 0,78 % o-phenanthroline solution (in the ratio 1:1). This mixture was centrifuged at 2,000g for 15 min at the room temperature. Further purification of precipitate was done by washing three times with addition of 0,9 % NaCl in volume equal to the initial volume of plasma. The concentration of SFMC was measured through the time record from the moment of addition of the reagent until the first plates of fibrin were precipitated. Measurements were carried out only to 150 seconds. Test was considered positive for appearance of visible light plates during the first 150 seconds. The time of the plates appearance was noted in seconds and concentration was calculated according the calibration chart [10].

For the next step SFMC were collected from the 1 ml of blood plasma of each pathology by incubation with 0,78 % o-phenanthroline per 5 min. Aliquots in volume 50 mkl of each tested fraction of SFMC were diluted 10 times by SDS-PAGE sample buffer and applied on in 8 % polyacrylamide gels in the presence of sodium dodecyl sulfate in denaturing conditions [11, 12]. Electrophoresis was done in the unit for vertical preparative disc electrophoresis (BioRad) in glass plates 1 mm thick in amperage 19 mA for concentrating and 35 mA for separating gels. Gels were stained with 0,125 % solution of coomassie G-250 in 25 % isopropanol and 10 % acetic acid. Washing of excess paint was performed using a 0,8 % solution of acetic acid.

Electrophoresis was followed by Western Blotting. 10 time diluted aliquots of SFMC were transferred into Amersham Hybond-C Membrane under standard conditions.

Western blot detection was performed using primary polyclonal anti-α-FDP in ration 1:2000 (Shijin International, Mongolia). Corresponding secondary antibodies (Sigma, USA) horseradish peroxidase-conjugated antibody in ratio 1:1500 were detected by 3,3'-diaminobenzidine (DAB) substrate 5 mg/ml with 30 % H_2O_2.

Statistical analysis was performed using statistical analysis applications of Microsoft® Excel. To assess intergroup differences the parametric Student test was used. The difference between the parameters was considered statistically significant at $p<0,05$. Analysis of electrophoregrams was performed by the scanning of the computer program TotalLab 2.01.

Results. SFMC fractions were obtained from each tested group: healthy donors, patients with acute AIS, acute CIS and the same group of patients one year past AIS or CIS were diagnosed.

The first stage of this study was to determining of the concentration of SFMC by o-phenanthroline test in blood plasma of patients with cardioembolic and atherothrombotic ischemic stroke in the acute phase of the disease and one year after. The data shown in a Table 1 summarize the dynamic changes of concentration of the SFMC. It was shown that both acute stroke fractions was significantly higher comparing to the healthy donors value and was equal 33,4±1,5 mg/l for AIS patients and 36,9±1,2 mg/l for CIS patients. Also, studies indicate that one year past acute phase this index was equal 8,1±2,4 mg/l for AIS and 13,8±2,6mg/l for CIS patients. Accordingly, concentrations of soluble fibrin monomer complexes become close to physiological one year past ischemic stroke.

Table 1. **Concentration of soluble fibrin monomer complexes in blood plasma of the patient with stroke**

Tested group	Concentration of SFMC (mg/l)
Healthy donors	2,0
AIS acute	33,4±1,5
AIS year past	8,1±2,4
CIS acute	36,9±1,2
CIS year past	13,8±2,6

At the second stage 8 % SDS-PAGE was performed. Our study showed presence of proteins with molecular weight from 20 kDa to higher that 330 kDa. Proteins with Mr about 330 kDa and higher are the soluble fibrin monomer complexes. It was also discovered fractions with Mr lover than 300 kDa which could witness about splitting by plasmin.

The next task of our work the qualitative composition of the SFMC fraction was determined through Western Blot analysis (Fig. 2). It was shoved that o-phenanthroline method of SFMC separation is accompanied by the additional precipitation of fibrinogen and fibrin fragments. The presence of SFMC with molecular weight higher 330 kDa as well as some fibrin and fibrinogen fragments with Mr corresponded to X 240 kDa, DD 180 kDa and Y 150 kDa were detected in healthy donors. Acute AIS fraction had the same content. The higher diversity of the proteins was observed in acute CIS and both year past stroke fractions. Molecular weights of the proteins from 10 to higher 330 kDa in these fractions were detected. It could be explained by activation of the fibrinolysis one year past ischemic stroke attack. Moreover the presence of fibrin and fibrinogen degradation products in the acute CIS fraction and absence of this fragments in acute AIS fractions showed the shifted it time fibrinolysis for AIS.

It is well known that fibrinogen is a 330-kDa soluble plasma protein. Fragmentation of fibrinogen occurs upon cleavages within the α-chain to release the αC fragments, thereby producing fragment X (260 kDa). Further cleavage of fragment X in the α-, β-, and γ-chains between the two disulfide rings in one half of the molecule produces fragment Y (160 kDa) and fragment D (100 kDa). Thus thrombin-treated fibrin fragments were noticed in each tested fractions. Further cleavage of fragment Y produces a second fragment D and fragment E (60 kDa) which were not noticed in healthy donors and acute AIC fractions.

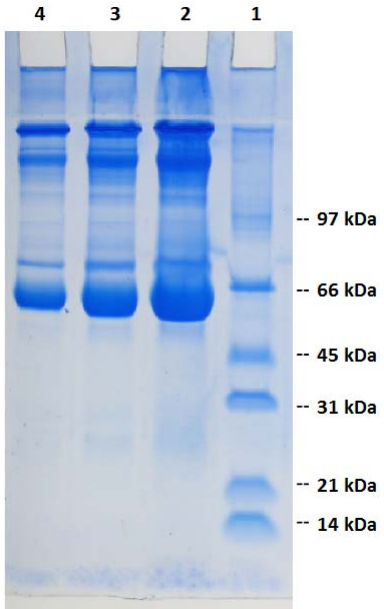

Fig. 1. SDS PAGE image of the fraction of soluble fibrin monomer complexes obtained from:
1. Molecular weight ladder; 2. Patients with AIS; 3. Patients with CIS; 4. Healthy donors

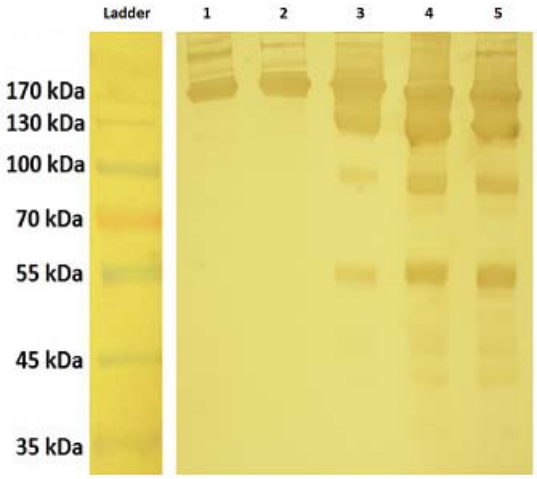

Fig. 2. Western Blot image of fraction of the soluble fibrin monomer complexes obtained from:
1. Healthy donors; 2. Patients with acute AIS; 3. Patients with acute CIS;
4. Patients with year past AIS; 5. Patients with year past CIS

Tables 2. **Content of fraction of soluble fibrin monomer complexes in plasma of ischemic stroke patients**

Healthy donors	AIS		CIS	
	Acute	Year past	Acute	Year past
>330	>330	>330	>330	>330
260	260	260	260	260
250	250	250	250	250
220	220	220	220	220
195	195	195	195	195
160	160	160	160	160
130	130	130	130	130
100	100	100	100	100
-	-	80	80	80
-	-	60	60	60
-	-	-	-	-
-	-	45	45	45
-	-	35	35	35
-	-	-	25	25
-	-	-	10	10

Conclusion. It was shoved that o-phenanthroline method of the detection of SFMC concentration is accompanied by the additional precipitation of fibrinogen and fibrin fragments. The presence of hipercoagulation marker as soluble fibrin monomer complexes in increased concentration in blood plasma of acute stroke patients was shown. This is evidence of coagulation activation. Nevertheless the concentrations of SFMC become more close to physiological one year past ischemic stroke. Instead of the concentration, the qualitative content of the year post stroke SFMC fraction was characterized by the higher diversity in comparison with acute fraction both subtypes of ischemic stroke. It could be explained by activation of the fibrinolysis one year past ischemic stroke attack. Moreover the presence of fibrin and fibrinogen degradation products in the acute CIS fraction and absence of this fragments in acute AIS fractions showed the shifted in time of the fibrinolysis process for AIS.

References

1. *GBD 2013 Mortality* and Causes of Death Collaborators. A systematic analysis for the Global Burden of Disease Study 2013. – Lancet. – 2015. – Vol. 385. – P. 117–71.
2. *Adamson J.* Is stroke the most common cause of disability? / J. Adamson, A. Beswick, S. Ebrahim // Stroke Cerebrovasc Dis. – 2004. Vol. 13. – P. 171–177.
3. *Wolfe C.D.A.* The impact of stroke / C.D.A. Wolfe // Brit.Med.Bull., 2000. – Vol. 56, N 2. – P. 275-286.
4. *Swarowska M.* Hyperfibrinogenemia predicts long-term risk of death after ischemic stroke / M. Swarowska, A. Polczak, J. Pera et al. // J. Thromb. Thrombolysis, 2014. – Vol. 38. – P. 517–521.
5. *Sanjeev P.* Overview of the coagulation system / P. Sanjeev, R. Saroa, A. Palta // Indian. J. Anaesth., 2014. – Vol. 58, N 5. – P. 515–523.
6. *Tynngerd N.* Assays of different aspects of haemostasis – what do they measure? / N. Tynngerd, T. Lindahl, S. Ramstrum // Thromb J., 2015. – Vol. 13. – P. 8.
7. *Johansson P.I.* Management of major blood loss: an update. / P.I. Johansson, S.R. Ostrowski, N.H. Secher // Acta Anaesthesiol Scand., 2010. – Vol. 54. – P. 1039–49.
8. *Clinical* implication of plasma level of soluble fibrin monomer-fibrinogen complex in patients with abdominal aortic aneurysm / A. Hosaka et al. // J. Vasc. Surg., 2005. – Vol. 42, № 2. – P. 200–205.
9. *Momot A.* Methodology and clinical significance of paracoagulation phenantroline test. / A. Momot, V. Lakomov, S. Barkagan // The wedge. laboratory diagnosis, 1996. – Vol. 4. – P. 17–20.
10. *Takahashi D.* Evaluation of hypercoagulability using soluble fibrin monomer complex in sick newborns / D. Takahashi // Pediatr. Int., 2013. – Vol. 55, № 2. – P. 151–156.
11. Cleveland D. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. / Fischer S, Kirschner M, Laemmli UK.// Journal of Biological Chemistry. – 1977. Vol. 252. N. 3. – P. 1102-1106.
12. Weber K. The reliability of molecular weight determinations by dodecyl sulfate-polyacriylamide gel electrophoresis./ Osborn M. // The Journal of Biological Chemistry. – 1969. Vol. 244. N.16. – P. 4406–4412.

References (Scopus)

1. GBD 2013 Mortality and Causes of Death Collaborators. A systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015; 385: 117–71.
2. Adamson J. Beswick A, Ebrahim S. Is stroke the most common cause of disability? Stroke Cerebrovasc Dis. 2004;13: 171–177. English.
3. Wolfe C.D.A. The impact of stroke. Brit.Med.Bull. – 2000; 56(2). 275-286. English.
4. Swarowska M., Polczak A., Pera J., Klimkowicz-Mrowiec A., Slowik A., Dziedzic T. Hyperfibrinogenemia predicts long-term risk of death after ischemic stroke. J. Thromb. Thrombolysis. 2014; 38: 517–521. English.

5. Sanjeev Palta, Richa Saroa Anshu Palta Overview of the coagulation system. Indian J Anaesth. 2014 Sep-Oct; 58(5): 515–523. English.
6. Nahreen Tynngerd, Tomas L Lindahl, Sofia Ramstrцm Assays of different aspects of haemostasis – what do they measure? Thromb J. 2015; 13: 8. English.
7. Johansson PI, Ostrowski SR, Secher NH. Management of major blood loss: an update. Acta Anaesthesiol Scand. 2010;54:1039–49. English.
8. A. Hosaka et al. Clinical implication of plasma level of soluble fibrin monomer-fibrinogen complex in patients with abdominal aortic aneurysm. J. Vasc. Surg. 2005; 42(2): 200 – 205. English.
9. Momot A, Lakomov V, Barkagan S. Methodology and clinical significance of paracoagulation phenantroline test. The wedge. laboratory diagnosis. 1996;4:17-20. English.
10. D. Takahashi et al. Evaluation of hypercoagulability using soluble fibrin monomer complex in sick newborns. Pediatr. Int. 2013; 55(2): 151 – 156. English.
11. Cleveland D, Fischer S, Kirschner M, Laemmli UK. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. Journal of Biological Chemistry. 1977; 252(3):1102-1106. English.
12. Weber K, Osborn M. The reliability of molecular weight determinations by dodecyl sulfate-polyacriylamide gel electrophoresis. The Journal of Biological Chemistry. 1969;244(16):4406–4412. English.

Submitted on 20.12.16

Т. Катрій, асп., Н. Шабанова, маг., С. Остапчук, студ., О. Савчук, д-р біол. наук
Київський національний університет імені Тараса Шевченка, Київ

ХАРАКТЕРИСТИКУ РОЗЧИННИХ ФІБРИН-МОНОМЕРНИХ КОМПЛЕКСІВ У ХВОРИХ ІШЕМІЧНИМ ІНСУЛЬТОМ У ГОСТРУ ФАЗУ ТА ЧЕРЕЗ РІК ПІСЛЯ ПЕРЕНЕСЕНОЇ ГОСТРОЇ ФАЗИ

Показано, що о-фенантроліновий метод визначення концентрації розчинних фібрин мономерних комплексів супроводжується додатковим осадженням фрагментів фібриногену та фібрину у плазмі крові. Доведено, що за атеротромботичного та кардіоемболічного підтипів ішемічного інсульту в гострій фазі захворювання у плазмі крові присутні висококонцентровані розчинні фібрин-мономерні комплекси, що є показником високої активності коагуляційної ланки системи гемостазу. Проте їх концентрація повертається до норми через один рік після перенесеної гострої фази. На відміну від концентрації, її якісний вміст для обох підтипів ішемічного інсульту характеризується більшою різноманітністю через рік після перенесеної гострої фази. Присутність фрагментів фібриногену та фібрину в обох річних фракціях інсультів доведено, що є свідченням активації фібринолітичної ланки гемостазу через рік після перенесеного захворювання. Наявність фрагментів фібриногену і фібрину в гостру фазу кардіоемболічного ішемічного інсульту та відсутність їх у гостру фазу атеротромботичного ішемічного інсульту вказує на відсутність якісної відповіді системи фібринолізу в гостру фазу атеротромботичного ішемічного інсульту.

Ключові слова: розчинний фібрин мономерних комплексів, ішемічний інсульт, гемостаз.

Т. Катрий, асп., Н. Шабанова, маг., С. Остапчук, студ., А. Савчук, д-р биол. наук,
Киевський национальный университет имени Тараса Шевченка, Киев, Украина

ХАРАКТЕРИСТИКА РАСТВОРИМЫХ ФИБРИН-МОНОМЕРНЫХ КОМПЛЕКСОВ У БОЛЬНЫХ ИШЕМИЧЕСКИМ ИНСУЛЬТОМ В ОСТРУЮ ФАЗУ И ЧЕРЕЗ ГОД ПОСЛЕ ПЕРЕНЕСЕННОЙ ОСТРОГОЙ ФАЗЫ

Было показано, что о-фенантролиновый метод определения концентрации растворимых фибрин мономерных комплексов сопровождается преципитацией фрагментов фибриноген фибрина в плазме крови. Доказано, что атеротромботический и кардиоэмболический подтипы ишемического инсульта в острой фазе заболевания сопровождается появлением высокой концентрации растворимых фибрин-мономерных комплексов в плазме крови, что является показателем активации коагуляции. Однако их концентрация возвращается к норме через один год после острой фазы. В отличии от концентрации исследуемой фракции, ее качественный контент для обоих подтипов ишемического инсульта характеризуется большим разнообразием через год после перенесенной острой фазы по сравнению с аналогичной фракцией полученной в острую фазу. Наличие фрагментов фибриногена и фибрина в обеих годовых фракциях было показано, что является свидетельством активации фибринолиза через год после атаки ишемического инсульта. Кроме этого присутствие фрагментов фибриногена и фибрина в острой фазе кардиоэмболического ишемического инсульта в отличии от острой фазы атеротромботического ишемического инсульта говорит об отсутствии качественного ответа системы фибринолиза в острую фазу атеротромботического ишемического инсульта.

Ключевые слова: растворимый фибрин мономерных комплексов, ишемический инсульт, гемостаз.

УДК 57.089.7

Є. Опейда, асп., М. Рудик, канд. біол. наук,
В. Святецька, інж. 1-кат., Л. Сківка, д-р біол. наук,
Київський національний університет імені Тараса Шевченка, Київ, Україна
О. Федорчук, канд. біол. наук,
Інститут експериментальної патології, онкології та радіобіології імені Р.Є. Кавецького НАН України, Київ, Україна

РЕАКЦІЯ ЛІМФОЇДНИХ ОРГАНІВ ЩУРІВ НА РІСТ ГЛІОМИ С6

Дослідження реакції периферичних організованих лімфоїдних структур на присутність злоякісної пухлини в мозку відкриває перспективи застосування імунотерапевтичних підходів до лікування цього захворювання, спрямованих на активацію презентації пухлинних антигенів поза зоною росту злоякісного новоутворення. Метою роботи була оцінка вагових індексів і клітинності лімфоїдних органів за умов росту гліоми С6 у щурів. Встановлено, що ріст гліоми С6 супроводжується зміною клітинності органів лімфатичної системи. Зареєстровані зміни вказують на ймовірність презентації антигенів гліоми С6 у периферичних організованих лімфоїдних утвореннях, а також на негативний вплив росту гліоми С6 на гомеостаз тимусу та селезінки.

Ключові слова: лімфоїдні органи, гліома, клітинність органу, ваговий індекс.

Вступ. Тривалий час загально визнаним вважався постулат про те, що мозок є "імунологічно привілейованою" тканиною, ізольованою від імунної системи гемато-енцефалічним бар'єром. Відсутність лімфатичних судин у мозку, згідно цього постулату, унеможливлює транспорт мозкових антигенів у периферичні організовані лімфоїдні структури [1]. Наразі відомо, що розчинні антигени і клітини з ЦНС здатні потрапляти у периферичні лімфоїдні органи шляхом дренажу тканинної та спинномозкової рідини назальними та шийними лімфовузлами. З кров'ю мозкові антигени можуть також потрапляти у селезінку [2].

Реакція організованих лімфоїдних структур на присутність злоякісної пухлини у мозку є важливим питанням фундаментальної і прикладної імунології. Оцінка вагових та клітинних показників лімфоїдних органів ві-