CLINICAL AND LABORATORY FEATURES OF HEMOSTATIC DISORDERS IN PATIENTS WITH RETINAL VEIN OCCLUSION

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The number of patients with retinal venous occlusions is increasing, especially among young people. Often, they have revealed a genetic predisposition to thrombosis. Risk factors for thrombosis are genetic resistance to activated protein C (RAPC), genetic defect in factor V (FV Leiden) and the presence of lupus anticoagulant (LA). In this study we analyze the dependence of the various parameters of hemostasis in patients with retinal vein occlusion (RVO) on the background of FV Leiden mutation and LA. A total of 150 patients (150 eyes) with RVO (mean age — 42 ± 10 years) were examined and divided into three groups. Group 1: patients with RVO, FV Leiden and LA (n = 12); group 2: patients with RVO and FV Leiden (n = 11) without LA; group 3: patients with RVO without FV Leiden and LA, selected from remaining 107 people for a comparable number of groups (n = 30). The control group was 50 people without RVO, but with hypertension. It was shown that RAPC index in patients with FV Leiden mutation and the LA has the less value (0,6 ± 0,01) on comparison to patients with RVO (1,50 ± 0,18) (p <0,05). They also have enhanced V, VIII and von Willebrand factors and intravascular platelet activity. LA exacerbates endotheliosis in the microvasculature of the retina and in combination with FV Leiden mutation increases the thrombogenesis, participating in the pathogenesis of ischemic thrombosis of central retinal vein and its branches, which clinically manifested as retinal thrombo-hemorrhagic syndrome. The hemostasis regulation genes polymorphisms detection (as well as lupus anticoagulant detection) is recommended to clarify the diagnosis and selection of adequate therapy.

Keywords: retinal vein occlusion, activated protein C, resistance, FV Leiden mutation, lupus anticoagulant

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ОСОБЕННОСТИ КЛИНИКО-ЛАБОРАТОРНОЙ ДИАГНОСТИКИ НАРУШЕНИЙ В СИСТЕМЕ ГЕМОСТАЗА У ПАЦИЕНТОВ С ОККЛЮЗИЕЙ ВЕН СЕТЧАТКИ

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Растет число пациентов с венозными ретинальными окклюзиями, особенно среди среди молодых людей. Зачастую у них выявляется генетическая предрасположенность к тромбозу. Факторами риска возникновения тромбоза являются наследственная резистентность к активированному протеину С (РАПС), генетически обусловленный дефект фактора V (FV Leiden) и присутствие в организме волчаночного антикоагулянта (ВА). В исследовании была изучена зависимость различных параметров гемостаза у пациентов с окклюзией вен сетчатки (ОВС) при наличии у них мутации FV Leiden и ВА. Обследовали 150 пациентов (150 глаз) с ОВС (средний возраст — 42 ± 10 года), затем разделили их на три группы. Группа 1: пациенты с ОВС, FV Leiden и ВА (n = 12); группа 2: пациенты с ОВС и FV Leiden (n = 11); группа 3: пациенты с ОВС без FV Leiden и ВА, отобранные из 107 человек для сопоставимой численности групп (n = 30). В контрольную группу включили 50 человек без ОВС, но с гипертонической болезнью. Показано, что индекс РАПС у пациентов с мутацией FV Leiden и ВА имеет наименьшее значение (0,6 ± 0,01) при сравнении с пациентами только с ОВС (1,50 ± 0,18) (p <0,05). У них также повышена активность факторов V, VIII и Виллебранда и внутрисосудистая активность тромбоцитов. ВА усугубляет эндотелиоз в микроциркуляторном русле сетчатки, а в сочетании с мутацией FV Leiden усиливает тромбогенез, участвуя в патогенезе ишемического тромбоза центральной вены сетчатки и ее ветвей, что клинически проявляется ретинальным тромбогеморрагическим синдромом. Для уточнения диагноза и выбора адекватной терапии рекомендуется определение полиморфизмов генов, участвующих в регуляции гемостаза, и присутствия волчаночного антикоагулянта в организме пациента.

Ключевые слова: окклюзия вен сетчатки, активированный протеин С, резистентность, мутация FV Leiden, волчаночный антикоагулянт

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Retinal vein occlusion (RVO) accounts for up to 60 % of acute vascular disorders of the eye; it ranks second to diabetic retinopathy in terms of severity of damage to the retina and carries a poor prognosis [1, 2]. Retinal vein thrombosis often precedes the onset of such life threatening conditions as acute myocardial infarction and stroke. RVO is traditionally seen as imbalance between thrombogenic and antithrombogenic factors. In the recent years, the number of patients with retinal vein occlusion has been increasing, especially among young and working-age individuals with genetic predisposition to thrombosis [3].

Thrombophilia is susceptibility to thrombosis associated with inherited or acquired defects of procoagulant and anticoagulant pathways. Among the risk factors for venous thrombosis are inherited resistance to activated protein C (activated protein C resistance, APCR) and a mutant variant of factor V (factor V Leiden, FV Leiden). Activated protein C breaks down the mutant factor V more slowly than it occurs in healthy individuals, which increases the rate of thrombin production and under certain conditions may lead to thrombosis at any age. The nature of APCR can be genetic associated with FV Leiden or acquired associated with the presence of antiphospholipid antibodies and the effects of oral contraceptives [4–6].

Antiphospholipid antibodies (APA) alter the homeostatic regulation of blood coagulation. The exact mechanism of thrombosis involving APA (in particular, the lupus anticoagulant, LA) has not been identified yet. The prothrombotic mechanism of APA action is putatively based on the inhibition of endogenous anticoagulant pathways: reduced antithrombotic potential of the vessel wall and impaired activity of natural coagulants cause hypercoagulation [7–11].

Hemostatic defects and their clinical manifestation in patients with RVO who also have FV Leiden and LA are understudied. The aim of this work was to study some aspects of clinical laboratory testing for these disorders in patients with RVO who have FV Leiden alone or a combination of FV Leiden and LA.

METHODS

The study included 63 male and 87 female patients (a total of 150 individuals or 150 eyes) with retinal vein occlusion. Mean age was 42 ± 10 years. Branch retinal vein occlusion was detected in 78 patients (52 %), central retinal vein thrombosis was detected in 72 patients (48 %); 56 patients had hypertensive disease; 30 patients were diagnosed with coronary artery disease; 15 had varicose veins of the lower extremities. The length of the observation period varied from 2 weeks to 2 years.

The patients were divided into 3 groups. Group 1 included 12 patients with both FV Leiden and LA; group 2 included 11 individuals with FV Leiden; group 3 included 107 patients with RVO and without activated protein C resistance. In group 3, we analyzed data from 30 patients to make sample sizes comparable. The control group included 50 individuals without RVO who had hypertensive disease, no signs of systemic or autoimmune damage to the connective tissue, no coronary artery disease, cancer, or severe chronic infections. Male to female ratio in the control group was 20 : 30.

The clinical diagnosis was made using standard ophthalmic techniques: a visual acuity test, ocular tonometry, perimetry, direct ophthalmoscopy, and a number of specific techniques, such as the examination of the ocular fundus using a Goldmann lens, fundus fluorescein angiography, optical coherence tomography of the retina, computed perimetry.

To study the hemostatic system, automated coagulation screening was performed. A number of measurements were taken, including determination of von Willebrand factor (vWF), antithrombin III, activated protein C, and coagulation factor VIII activities; determination of factor V levels in blood plasma, levels of soluble fibrin monomer complexes (SFMC) and fibrinogen (the Clauss assay); resistance of factor V to activated protein C (APCR index).

The presence of the lupus antigen was detected by venom tests and confirmed by donor plasma and phospholipid tests using reagent kits by Technology-Standard (Russia) and Instrumentation Laboratory (Italy). Six weeks later, the tests were repeated.

Factor V Leiden and other polymorphisms responsible for susceptibility to thrombophilia were detected using real time PCR.

For this study we used the CL4 homeostasis analyzer (Behnk Elektronik, Germany) and a platelet aggregometer (Research and Production Company Biola, Russia). All tests were carried out at the facilities of the Laboratories for Hemostasis and Genetics (Kemerovo Regional Clinical Hospital).

Statistical analysis was performed using Statistica 6.0 software by StatSoft, USA. To describe the groups, we used the mean and the standard error of the mean. The groups were compared using Student's T-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Of 150 patients with RVO, the lupus anticoagulant was found in 32 (12 %) individuals; 20 of them did not have FV Leiden, while 12 did. There were 11 (7.3 %) patients with RVO who had only FV Leiden. The hemostatic analysis (see the Table) demonstrated that SFMC levels in group 2 (mutant FV) and group 1 (FV Leiden + LA) were increased by 20 and 15 %, respectively, compared to group 3 (no FV Leiden or LA). Fibrinogen levels in groups 1 and 2 were by 37.5 and 20.0 % higher than in group 3. In group 1, intravascular platelet activation was more marked than in group 2: the total sum of activated platelets was increased by 16.2 % and the number of platelets involved in aggregation was by 26.3 % higher. Intravascular platelet activity was also increased in group 3, but it was less conspicuous than in patients with FV Leiden and no LA: 10.7 % vs. 16.4 %, respectively. Factor V activity was increased by 25 % in groups 1 and 2, compared to the controls. Groups 1 and 2 demonstrated a statistically significant 2.3- and 2.6-time increase in the activity of coagulation factor VIII, respectively, compared to the controls. Compared to group 3, this activity was by 27 and 30 % higher, respectively. Von Willebrand factor also exhibited increased activity in all three groups of patients with RCO, in contrast to the controls: by 55, 70 and 30 % in groups 1, 2 and 3, respectively. It proves that in retinal vein occlusion, the main role is played by the venous endothelium. However, increased activity of vWF in groups 1 and 2 was more conspicuous compared to group 3: by 16.0 and 23.5 %, respectively.

Protein C activity was more conspicuous in patients from groups 2 and 3 who had RVO in comparison with the controls: by 36.5 and 39.4 %, respectively. APCR was reduced in groups 1 and 2 by 64.0 and 58.9 %, respectively, in comparison with healthy individuals. In group 3 APCR was by 53.6 and 46.4 % higher than in groups 1 and 2, respectively. In patients with FV Leiden, the APCR index value was lower if LA was present.

In all groups, patients with RVO had elevated fibrinogen (inflammation protein) levels in comparison with healthy

individuals: they were increased by 56.3, 43.3 and 25.0 % for groups 1, 2 and 3, respectively. In groups 1 and 2 this index was 1.5- and 1.2-times higher, respectively, than in group 3.

Clinical test revealed that patients from groups 1 and 2 had microcirculatory disorders accompanied by vaso-occlusive processes in the ocular fundus. Fundus fluorescein angiography proved the presence of retinal leakage and local ischemia (both in the retinal center and on the periphery) and the absence of capillary perfusion (Fig. 1, 2). All patients had ischemic occlusion of the central retinal vein or its branches accompanied with marked cystoid macular edema (mean macular thickness measured by OCT was 790 ± 20 μm). Persistent cystoid macular edema was observed in 8 % of patients.

DISCUSSION

Increased activity of factor V demonstrated by groups 1 and 2 can be explained by the presence of FV Leiden in patients' blood. Activated protein C breaks it down more slowly, so the mutant factor V accumulates in blood. Factor V participates in the activation of prothrombinase complexes (XIa, VIIIa, vWF) mediating prothrombin conversion to thrombin. Protein C inactivates the mutant factor V slowly, which results in the prolonged factor VIII activity and can explain its elevated levels.

Antiphospholipid antibodies of the lupus type interact with the components of the vascular wall and endothelium; this stimulates the synthesis of vWF and factor VIII. LA activates platelets through membrane receptors, mediates the release of histamine, serotonin and platelet factors 3 and 4 from platelets, inhibits the synthesis of prostacyclin (a powerful inhibitor of platelet aggregation and a vasodilator), inhibits thrombomodulin and proteins C and S activities and thus provokes hypercoagulation. LA disrupts normal hemostatis and increases consumption of procoagulants and natural anrticoagulants. It also maintains aseptic inflammation in the venous wall and triggers thrombus formation in the retina.

CONCLUSIONS

Clinical and laboratory hemostatic tests performed in patients with retinal vein occlusion revealed that in the presence of FV Leiden, the activity of factors V, VIII and von Willebrand factor is increased and the resistance to activated protein C is reduced; in the presence of lupus anticoagulant significant aggravation of endotheliosis, thrombinemia and thrombus formation in the retinal microvasculature is observed manifested through thrombohemorrhagic complications. In patients with FV Leiden, the APCR index reduction is more marked in the presence of LA.

APC-resistance, both inherited (FV Leiden) and acquired (LA), disrupts the regulation of hemostasis in patients with RVO and therefore one of the clues to the understanding of the ischemic occlusion of the central retinal vein.

The obtained results prove that detection of polymorphisms participating in the hemostatic regulation, as well as detection of LA, is essential in patients with RVO, as it helps to clarify the nature of retinal thrombosis and can be used in the continuous monitoring of hemostasis in such patients aiming to deliver timely diagnosis and plan an adequate combined anticoagulation, platelet antiaggregation, antioxidant and afferent therapy.

Homeostasis in patients with retinal vein occlusion depending on the presence of FV Leiden and lupus anticoagulant (LA) in patients' blood

Parameter	Controls (n = 50)	Group 1: patients with RVO, FV Leiden and LA (n = 12)	Group 2: patients with RVO, FV Leiden and no LA (n = 11)	Group 1: patients with RVO, no FV Leiden and no LA (n = 30)
Factor V, %	100.0 ± 8.7	125.0 ± 4.3[a,c]	128.0 ± 4.3[a,c]	132.0 ± 3.9[a,c]
Factor VIII, %	102.0 ± 9.6	235.0 ± 2.2[a,b,c]	265.0 ± 4.3[a,b,c]	185.0 ± 6.6[a,b,c]
von Willebrand factor, %	100.0 ± 7.5	155.0 ± 5.8[a,b,c]	170.0 ± 9.0[a,b,c]	130.0 ± 12.3[a,c]
ACPR index	1.8 ± 0.02	0.65 ± 0.01[a,b,c]	0.74 ± 0.01[a,b,c]	1.40 ± 0.02[a,c]
C protein, %	104.0 ± 7.8	118.0 ± 4.7[a,b,c]	142.0 ± 5.1[a,b,c]	145.0 ± 6.7[a,c]
Antithrombin III, %	116.0 ± 11.7	155.0 ± 5.8[a,b,c]	118.0 ± 3.8[a,b,c]	122.0 ± 10.4[a,c]
Fibrinogen, %	3.2 ± 0.2	5.0 ± 0.3[a,c]	4.6 ± 0.2[a,c]	4.0 ± 0.2[a,c]
SFMC. mg%	4.6 ± 0.2	11.0 ± 0.5[a,c]	10.0 ± 0.5[a,c]	8.0 ± 0.5[a,c]
Total active platelet forms, %	12.8 ± 0.5	37.3 ± 1.7[a,b,c]	31.0 ± 1.1[a,b,c]	28.0 ± 2.1[a,c]
Number of platelets involved in aggregation, %	6.8 ± 0.4	9.9 ± 0.3[a,b,c]	7.1 ± 0.6[a,b,c]	6.1 ± 0.5[a,c]

a — p <0.05; comparison of groups 1, 2 and 3 with the controls;
b — p <0.05; group 1 compared to group 2;
c — p <0.05 groups 1 and 2 compared to group 3.

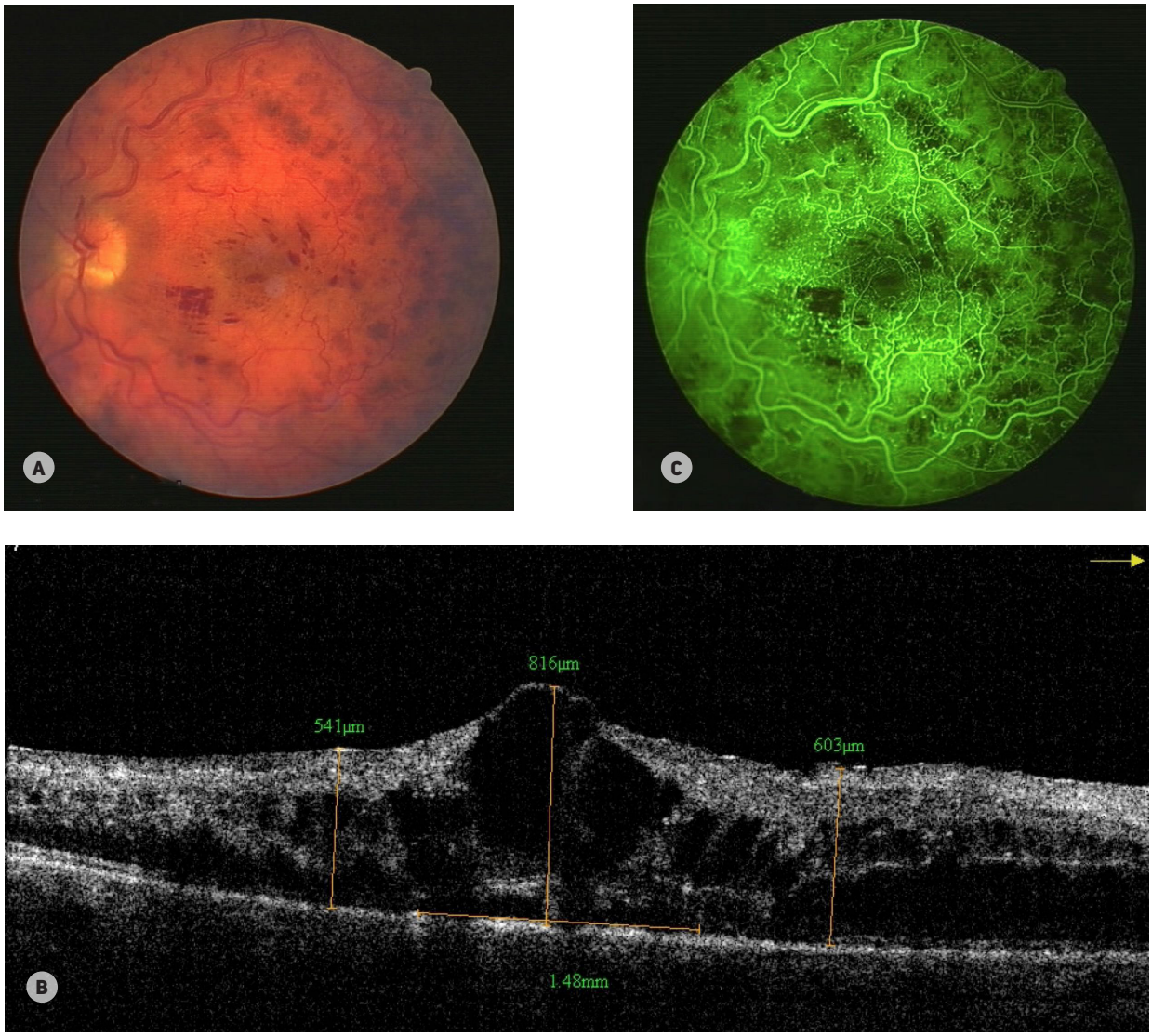

Fig. 1. Patient S., 38 years old. Diagnosed with central retinal vein occlusion; lupus anticoagulant and FV Leiden detected. **(A)** Conspicuous retinal hemorrhage syndrome. **(B)** Results of optical cogerence tomography. Diffuse cystoid macular edema; foveal thickness of 816 μm, peri- and parafoveal thickness of 541–603 μm; destroyed pigment epithelium and photoreceptor layer, d = 148 mm. **(C)** Fundus fluorescein angiography. Leakage from retinal vessels, cystoid macular edema, retinal ischemia

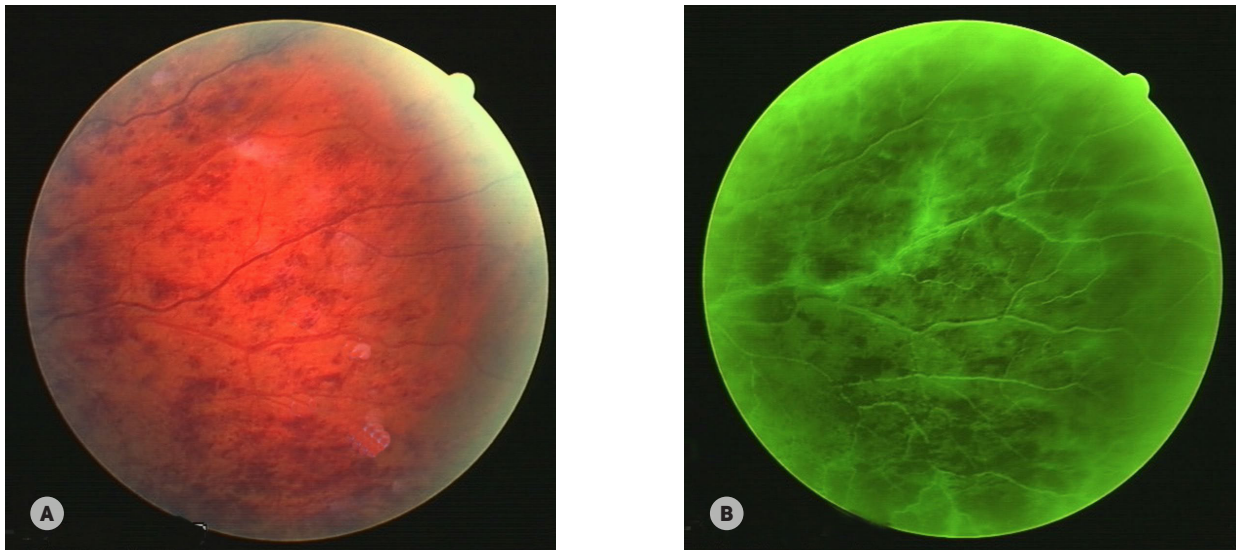

Fig. 2. Patient S., 38 years old. Diagnosed with central retinal vein occlusion; lupus anticoagulant and FV Leiden detected. **(A)** Medium periphery. Conspicuous retinal hemorrhage syndrome. **(B)** Fundus fluorescein angiography. Leakage from retinal vessels, cystoid macular edema, retinal ischemia

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