Research paper

New method in assessment of blood plasma coagulation balance

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ABSTRACT

Introduction: Thrombotic complications associated with disorders of hemostatic system are quite common among various categories of patients and require timely objective assessment of coagulation balance.

Aim: To develop an integrated index of assessment of blood plasma coagulation balance using the method of 'overall haemostasis potential,' and to test its practical value in clinical settings.

Material and methods: 179 patients were studied: 88 with chronic kidney disease (CKD) of VD stage, treated by program hemodialysis, and 91 with ischemic heart disease (IHD) after percutaneous transluminal coronary angioplasty (PTCA). The state of overall coagulation and fibrinolytic potentials was studied in all patients using the method of overall hemostasis potential (OHP). Based on this method, coagulation index (CI) was calculated and tested in such categories of patients.

Results and discussion: Patients with stage VD CKD demonstrated the tendency to hypocoagulation, while those with coronary heart disease (CHD) and PTCA – significant hypercoagulation, being indicated by CI index.

Conclusions: Patients with stage VD CKD have the tendency to hypocoagulation, while those with CHD and PTCA – to hypercoagulation. The integrated index suggested for assessment of coagulation potential – coagulation index – can adequately reflect coagulation balance disorders and considerably simplify their quantitative assessment.

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1. INTRODUCTION

According to literature data, overall hemostasis potential (OHP) of blood plasma is a modern approach to determine any changes in the delicate balance between the systems of coagulation and fibrinolysis.[1,2] The method is based on analysis of the curve of light absorption by the clot, which corresponds to the process of formation and destruction of the clot in plasma in the presence of thromboplastin and tissue plasminogen activator (t-Pa). The whole process of formation and destruction of fibrin clot is as follows: tissue factor contained in thromboplastin forms complex with factor VIIa, which activates factors X, IX and VII.[1,3,4] Activated factor Xa directly promotes the transition of prothrombin to thrombin, which is formed in relatively small concentrations and, in its turn, activates factors V, VIII, XI, followed by the formation of tenase (FIXa-FVIIIa) and prothrombinase (FXa-FVa) complexes on the phospholipid thromboplastin surface.[3,4] These complexes considerably accelerate activation of FX in FXa and, consequenly, the conversion of prothrombin to thrombin.[5,6] Subsequently, thrombin formed during activation of blood coagulation converts fibrinogen into fibrin, which forms a fibrin clot following polymerization. The fibrin clot sorbs plasminogen and its tissue activator (t-Pa) on the surface, which enhances the transition of plasminogen to plasmin leading to fibrin clot destruction.[1,3,6] Therefore, the area under the absorption curve will vary depending on the concentration of coagulants, anticoagulants or components of fibrinolysis (Figure 1).[1,2,7]
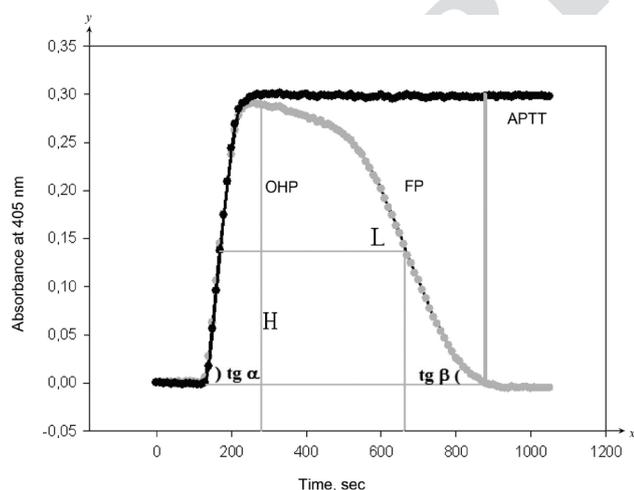

Figure 1. The curve of blood plasma coagulation initiated by APTT reagent in the absence of t-PA (dark curve color) and its presence (light curve color). Comments: t – lag period of plasma coagulation; FP (fibrinolytic potential) – area under the curve corresponding to the value of plasma fibrinolytic potential in ODU/s; OHP – area under the curve, which corresponds to the value of overall hemostasis potential in ODU/s; OHP + FP = area of CP (coagulation potential); H – maximum turbidity of the clot; tg α – rate of fibrin fibrils formation; tg β – rate of fibrin clot destruction; L – half-life time of plasma clot; APTT – activated partial thromboplastin time.

The informative value of absorption curve, both in vitro and in vivo – in blood plasma – is underlined in modern literature.[8] Thrombin is known to bind to thrombomodulin in the bloodstream and activate anticoagulant system, its major protein being protein C, which helps regulate thrombin levels and reduce the likelihood of thrombosis.[3,9–11] However, discrete assessment of total hemostatic potential and fibrinolytic potential (FP) complicates the use of those indices as a holistic integral component to quantify the degree of plasma coagulation imbalance.

2. AIM

To develop an integrated index of assessment of blood plasma coagulation balance using the method of OHP, and to test its practical value in clinical settings.

3. MATERIAL AND METHODS

After receiving an informed consent, 179 patients were studied during the period of 2016–2018: 88 patients (36 females and 52 males, aged 26 to 65 years) with chronic kidney disease (CKD) of VD stage, treated by program hemodialysis at Hemodialysis Center of Vinnytsia Regional Hospital named after M.I. Pirogov; and 91 patients (77 males and 14 females, aged 33 to 80 years) with ischemic heart disease (IHD) and percutaneous transluminal coronary angioplasty (PTCA), treated at Vinnytsia Regional Clinical and Diagnostic Center of Cardiovascular Pathology. Blood samples of patients and apparently healthy volunteers were placed in 3.8% sodium citrate (1 part of sodium citrate and 9 parts of blood, pH 7.4). In patients with stage VD CKD, blood sampling was performed before hemodialysis procedure and in those with coronary heart disease (CHD) – 6 months after angioplasty. Plasma of 23 healthy volunteers (10 females and 13 males, aged 24 to 60 years) served as the control.

Plasma was separated from blood cells by centrifugation at 3000 rpm for 20 minutes. The balance between blood coagulation and fibrinolysis systems was evaluated using OHP assay by the method based on interpretation of the curve of light absorption by the clot at 405 nm against time. The results were registered by microreader Multiskan EX (Thermo Scientific, Finland) plotting the curve, which showed the formation and destruction of the clot in blood plasma in the presence of thromboplastin (Sigma, USA) and tissue plasminogen activator.[1,8] Using a spectrophotometer, absorption of light at 405 nm by a fibrin clot formed in a spectrophotometric cuvette was recorded, after sequential addition of up to 0.05 M HEPES buffer, pH 7.4, containing 0.15 M NaCl, 70 μL of blood plasma, tissue plasminogen activator (t-PA, Boehringer Ingelheim) to a final concentration of 75 IU/mL and APTT reagent (Renam, Russia). Plasma coagulation process was initiated by addition of 25 mm $CaCl_2$. The final volume of reaction mixture was 300 μL. Overall hemostatic potential was characterized by the size of the area under the clot turbidity curve from the moment of

initiation of plasma coagulation to the moment of complete destruction of the clot in the presence of tissue plasminogen activator. The coagulation potential (CP) was estimated as the area under the clot formation curve in the absence of t-Pa. FP was the difference between the values of CP and OHP. All values were expressed in units of optical density multiplied by time in seconds (ODU/s).[7] Reaction mixtures without thromboplastin served as a control. The value of light amount absorbed by the clot at 405 nm in the control group was subtracted from the values of absorption in study patients.

Statistical processing of study results was performed by the methods of variation statistics using Student's t-test. Exclusion criteria were oncological diseases, persistent atrial fibrillation, acute coronary syndrome, stroke, liver cirrhosis, heart failure of functional class III–IV according to NYHA classification.

4. RESULTS

Since the resultant curve of coagulation potential contains information about the relationship between its main components, reflected in the size and lifetime of fibrin clot, and its integral index capable to reproduce the processes of formation and destruction of the clot is the surface area made by the curve, it is reasonable to present the whole process in the form of coagulation index (CI). This index should reflect the indices of overall coagulation and fibrinolytic potentials in the controls and in study patients. To calculate CI, first parameters of OHP and FP of study patients were compared with similar parameters in the control group, thus receiving indexes OHP (IOHP) and FP (FPI). Comparison of IOHP and FPI yielded CI which reflected the correlation between hemostasis parameters and the control, as well as between hemostasis parameters themselves. The proposed coagulation index is an integral component, which enables to quantify the degree of coagulation imbalance. Decrease of CI below 1 (control value is taken as 1) was interpreted as hypocoagulation, and its increase above 1 – as hypercoagulation. CI values exceeding 1 were registered in 57 (62.4%) patients with IHD and only 16 (18.2%) patients with stage VD CKD. It should be noted that average value of CI was 0.87 ± 0.08 in patients with stage VD CKD having the tendency to decrease as compared to the controls, while in patients with coronary heart disease and PTCA CI was significantly increased being 1.33 ± 0.08 ($P = 0.001$).

To prove the effectiveness of CI suggested, we present some examples of hemostasis status in patients of study groups.

4.1. Example 1

Patient D (No 4), 49 years. Diagnosis: IHD. Postinfarction myocardiosclerosis. Percutaneous coronary angioplasty (2015). Late restenosis/stent thrombosis.

Coagulation potential parameters of patient D are presented in Table 1.

Conclusion: Confirmed state of hypercoagulation (CI >1) with thrombotic complications. Figure 2 shows characteristic coagulation potential curve of patient D (No 4).

Table 1. Coagulation potential parameters of patient D.

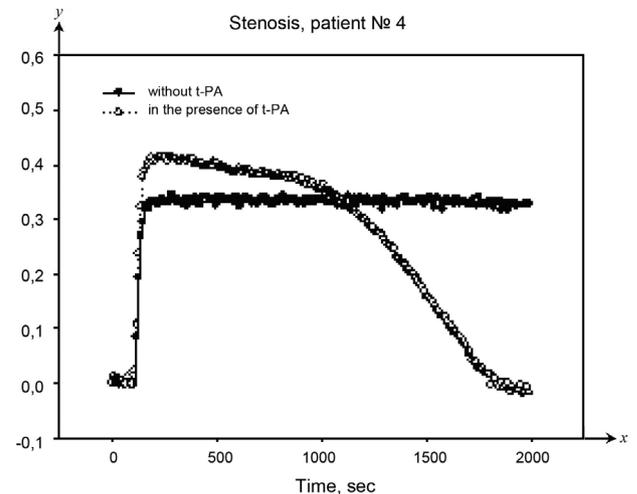| Study groups	OHP	FP	IOHP	FPI	CI
Control	209.8 ± 16.6	72.5 ± 5.8	–	–	1.0
Patient D	504.5	63.4	2.40	0.87	2.76

Figure 2. Patient D. Characteristic curve of coagulation potential for hypercoagulation status.

4.2. Example 2

Patient P (No 14), 59 years. Diagnosis: IHD. Class III exertional angina. Percutaneous coronary angioplasty (2015). Recurrent nasal bleeding.

Parameters of coagulation potential of patient P are presented in Table 2.

Conclusion: Confirmed state of hypocoagulation (CI < 1) with hemorrhagic complication. Figure 3 shows the characteristic curve of coagulation potential in patient P (No 14).

Table 2. Coagulation potential parameters of patient P.

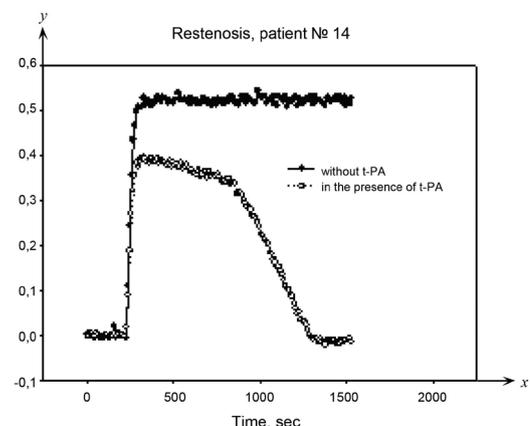| Study groups	OHP	FP	IOHP	FPI	CI
Control	209.8 ± 16.6	72.5 ± 5.8	–	–	1. 0
Patient P	298.7	238.0	1.42	3.28	0.43

Figure 3. Patient P. Characteristic curve of coagulation potential for hypocoagulation state.

Table 3. Coagulation potential parameters of patient S.

Study groups	OHP	FP	IOHP	FPI	CI
Control	209.8 ± 16.6	72.5 ± 5.8	–	–	1. 0
Patient S	287.9	70.5	1.37	0.97	1.41

4.3. Example 3

Patient S. (No 14/2) CKD VD stage. Chronic glomerulonephritis. Thrombophlebitis of the veins of lower extremities. Lymphostasis.

Parameters of coagulation potential of patient S. are presented in Table 3.

Conclusion: obtained coagulation index (CI = 1.41) verifies the state of hypercoagulation.

The examples presented demonstrate sufficient diagnostic value of the proposed integrated index – coagulation index[12].

5. DISCUSSION

According to study results, various degrees of coagulation system disorders were observed in two groups of study patients. Much lower proportion of hypercoagulation states in patients with stage VD CKD could be attributable to anticoagulant therapy (heparin), used during program hemodialysis.[13] By contrast, high proportion of hypercoagulation states in patients with coronary heart disease and PTCA could be associated with decreased activity of tissue plasminogen activator (t-PA) being characteristic of this category of patients.[3] It is decreased t-PA activity that leads to imbalance between the processes of coagulation and fibrinolysis.

Thus, the study conducted and clinical examples presented above provide strong evidence that integrated index CI developed and tested by the authors for assessment of coagulation system potential adequately reflects disturbances in coagulation balance as well as considerably simplifies their quantitative assessment.

6. CONCLUSIONS

(1) Patients with stage VD CKD have a tendency to hypocoagulation, while those with coronary heart disease and PTCA – to hypercoagulation.
(2) The proposed integrated index of coagulation balance assessment in the form of coagulation index adequately reflects coagulation system status and simplifies its quantitative assessment.

Conflict of interest
The authors declare no conflict of interest.

Ethics
Ethics committee approval for this study was obtained (approval No 4/2020).

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