PREDICTORS OF THROMBOGENESIS BY THE STATE OF PRO- AND ANTICOAGULANT COMPONENTS OF HEMOSTASIS IN THE PATIENTS WITH STAGE VD CHRONIC KIDNEY DISEASE

CZYNNIKI PREDYKCYJNE TROMBOGENEZY W KONTEKŚCIE PRO- I PRZECIWZAKRZEPOWYCH ELEMENTÓW UKŁADU HEMOSTAZY U CHORYCH Z PRZEWLEKŁĄ CHOROBĄ NEREK W STADIUM VD

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ABSTRACT

Introduction: One of the major complications of stage V chronic kidney disease (CKD), treated by program hemodialysis, are hemostatic system disturbances resulting in thrombosis development. To detect early predictors of potential thrombosis in study category of patients is rather difficult because of inflammatory process, accumulation of antibodies, continuous damage of blood elements.

The aim: To estimate potential applicability of activators and inhibitors of thrombogenesis as thrombophilia markers in the patients with stage V CKD, treated by program hemodialysis.

Materials and methods: 88 patients (52 males and 36 females) with stage V CKD, treated by program hemodialysis, were studied. Hemostatic profile was estimated in all the patients (soluble fibrin, D-dimer, protein C, fibrinogen).

Results: Two thirds of the patients with stage V CKD, treated by program hemodialysis, had significant increase in soluble fibrin and fibrinogen concentration against the background of decrease of natural anticoagulant - protein C and disparate response of D-dimer (tendency to decrease). It was established that comprehensive estimation of activators and inhibitors of thrombogenesis can become an indicator of thrombophilias in the patients with stage V CKD, treated by long term hemodialysis.

Conclusions: The majority of patients (68.2%) with stage VD CKD, treated by long term hemodialysis, had significant increase of soluble fibrin level ($p < 0.02$) along with decreased D-dimer to borderline values. In general group of patients there was significant decrease of protein C ($p<0.05$) on the background of great increase of fibrinogen concentration in the majority of patients (62%) ($p < 0.001$). The females were found to have significant increase of D-dimer level ($p < 0.05$) along with the increase of soluble fibrin concentration. Comprehensive determination of activators and inhibitors of thrombogenesis can serve an indicator of thrombophilias in the patients with stage VD CKD, treated by program hemodialysis.

KEY WORDS: chronic kidney disease, hemodialysis, hemostasis, thrombogenesis

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INTRODUCTION

In 2014 WHO health care experts called kidney disorders the major noninfectious diseases of today's world (ERA–EDTA Registry, 2014) [1]. Official data show that not less than 10% of people worldwide have renal pathology. According to the figures provided by Lancet, mortality rate from chronic kidney diseases has increased by 82% worldwide during the period between 1990 and 2010, and now they take the third place by the rate of mortality increase after HIV/AIDS and diabetes [2].

Nowadays prevalence and incidence of chronic kidney disease (CKD), as well as the cases of its late stages are on increase. Significance of nephrological pathology is explained by the severity of kidney diseases, restrictions in life activities and early disability in active working age, being a great social and economic problem even in highly developed countries.

The terminal stages of CKD significantly affect the hemostatic system, because, on the one hand, the kidney produces pro- and anticoagulant factors (heparin, urokinase, tissue plasminogen activator, thromboplastin, coagulation factors VII,VIII, IX, X), and on the other hand – it can promote the production of antibodies to vascular endothelium, proteins C and S, thrombomodulin, heparin sulfate etc. Kidneys are able to absorb and catabolize Hageman coagulation factor (XII) and, partially, fibrinogen [3]. The importance of abnormalities of blood composition in the pathogenesis of secondary hypercoagulation states (in nephrology – glomerulonephritis, nephrotic syndrome and hemodialysis itself) is

Table I. Hemostatic profile in general group of patients with stage VD CKD

No	Hemostatic profile (n=88)	Control group (donors)	Patients with stage VD CKD	P
1	Soluble fibrin (μg/ml)	2,60±0,37	3,54±0,14	≤0,02
2	D-dimer (pg/ml)	95,0±32,0	75,95±10,35	≥0,05
3	Fibrinogen (mg/ml)	2,20±0,36	4,15±0,12	≤0,001
4	Protein C (%)	100,0±10,0	80,05±1,47	≤0,05

Table II. Levels of pro- and anticoagulant indices of hemostasis in the patients with stage VD CKD according to soluble fibrin concentration

No	Soluble fibrin levels (μg/ml)	n	Soluble fibrin (μg/ml)	D-dimer (pg/ml)	Fibrinogen (mg/ml)	Protein C (%)
1	Group 1 (up to 3,0 μg/ml)	28	2,49±0,063	49,23±5,13	3,96±0,21	79,17±2,50
2	Group 2 (3,1-3,9 μg/ml)	38	3,48±0,035	68,10±5,42	4,36±0,17	81,97±2,36
3	Group 3 (4,0 μg/ml and over)	22	5,14±0,221	114,67±22,67	4,2±0,27	81,11±3,75
4	General group	88	3,54±0,14	75,95±10,35	4,15±0,12	80,05±1,47
5	P		P1-2 ≤0,001 P1-3 ≤0,001 P2-3 ≤0,001	P1-2 ≤0,05 P1-3 ≤0,01 P2-3 ≤0,05	P1-2 ≥0,05 P1-3 ≥0,05 P2-3 ≥0,05	P1-2 ≥0,1 P1-3 ≥0,1 P2-3 ≥0,1

a debatable issue. Endothelial cells in the patients with renal failure are known to lose their antithrombogenic properties when they interact with pro-inflammatory antigens and endotoxins, considerably increasing the risk of thromboembolism development [3].

Some of the major complications of stage V CKD, treated by program hemodialysis, are hemostatic system disturbances resulting in thrombosis development [3]. Thrombophilic syndrome, being a process of intravascular thrombogenesis, is characterized by systemic activation of blood coagulation, and is not compensated by inner natural anticoagulant mechanisms [4]. To detect early predictors of potential thrombosis in the studied category of patients is rather difficult because of inflammatory process, accumulation of antibodies, continuous damage of blood elements and vascular access. Accordingly, evaluation of hemostasis status should be based on comprehensive analysis of blood indices, which provide objective characteristics of coagulation processes. Fibrinogen is well known to be the central enzyme of coagulation system, and activation of coagulation system leads to formation of fibrin in blood circulation with its subsequent degradation by plasmin. Soluble fibrin and D-dimer are the most significant molecular markers of those processes [5]. At the same time, the evaluation of coagulation inhibitor potential is essential to improve thrombosis risk prediction. Such natural inhibitor is protein C [6]. Soluble fibrin, D-dimer, fibrinogen and protein C values in the patients with stage V CKD, treated by program hemodialysis, were studied to estimate potential applicability of thrombogenesis activators and inhibitors as thrombophilia markers.

THE AIM

To estimate potential applicability of activators and inhibitors of thrombogenesis as thrombophilia markers in the patients with stage V CKD, treated by program hemodialysis.

MATERIALS AND METHODS

Blood sampling in the studied patients and plasma donors was taken on empty stomach, without tourniquet, into vacutainer containing 3.8% sodium citrate solution, and was mixed in the ratio 1:9. The vacutainer mixture was stirred for several times without shaking. Precipitation of cellular blood elements was performed by centrifugation with relative centrifugal force 1200-1400 g for 20 minutes. Blood plasma (supernatant) was transferred in Eppendorf polyethylene tube.

To estimate soluble fibrin (the marker of coagulation system activation) in the patients, we used two-site enzyme-linked immune-sorbent assay for quantitation of products formed after degradation of fibrin by plasmin. Monoclonal antibodies III-3b were used as "catch"-antibodies, and monoclonal antibodies II-4d – as "tag" antibodies [7]. D-dimer was estimated by enzyme immunoassay using monoclonal antibodies to D-dimer epitopes, which are formed only after degradation of unsoluble febrin by plasmin [8]. Protein C activity in blood plasma was estimated by its activation with copperhead snake venom (Agkistrodon halus) [9]. For this purpose 30 μl of blood plasma, 100 μl of protein C activator, 85 μl 0.05M Tris-HCI buffer (pH 7,4) containing 0,13 M NaCl and 35 μl 2мМ chromogenic substrate S2236,

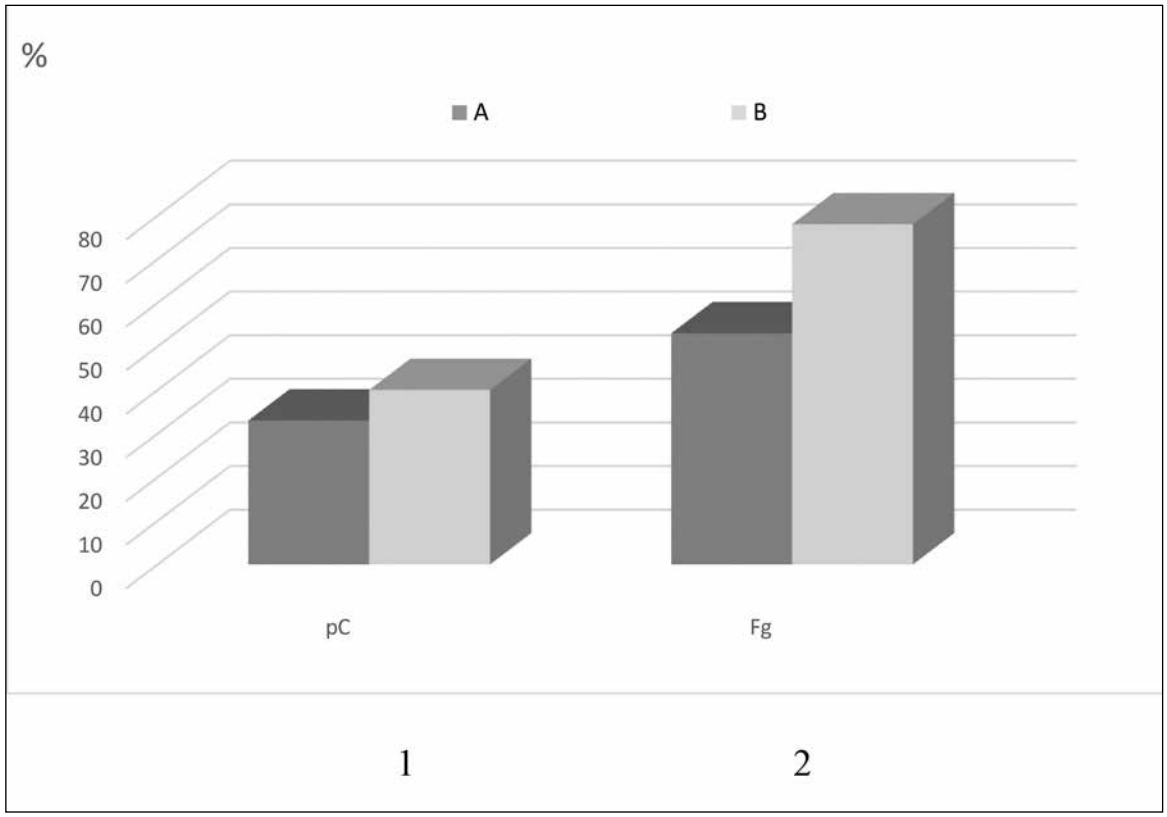

Fig. 1. Relationship between activity of protein C (pC) (A) and fibrinogen (Fg) (B) in blood serum of the patients with stage VD CKD according to soluble fibrin (sF) concentration: 1 – normal F contents; 2 – increased F contents.

were incubated at 37° C for 15 minutes. The number of degraded chromogenic substrate was determined by spectrophotometry with wavelength 405 and 492 nm on microplate spectrophotometer Thermo Multiskan EX, taking the absorption coefficient 1M of pNA solution at 405 nm to be 10500.

Serum fibrinogen contents was estimated using thrombin-like enzyme Antsistron-H obtained from copperhead snake (Agkistrodon halus) [10] by spectrophotometry. For this purpose 0,2 ml of studied plasma and 1,8 ml 0,1 M phosphate buffer (ph 7,0) were inserted into the glass tube with consequent addition of 0,1 Antsistron-H, containing 0,3 activity unit. The mixture was thoroughly stirred with lapped face glass spatula. After 30 minutes of incubation at 37° C, formed fibrin clot was retracted by rolling-up on the spatula, pressing the fluid over the tube walls. The clot on the spatula was then washed in cold solution of sodium chloride, and superficial fluid was removed from the clot by light touch to filter paper. Calculation was done by the following formula:

F = (E280 – E320) x 255/15,06 where

F – concentration of fibrinogen in blood plasma, g/l;

225 – coefficient for calculation of fibrinogen contents in the sample volume to its plasma concentration;

15,06 – coefficient of absorbing extinction of 1% fibrin solution in acid media at wavelength 280 nm.

Statistical processing of study results was performed by the methods of variation statistics.

RESULTS

According to study results, the patients of the general group (n=88) (52 males and 36 females) demonstrated significant ($p < 0,02$) increase of soluble fibrin level compared to the control group – 3,54±0,14 mkg/ml (Table I). It should be noted, that no proportional increase in D-dimer was noted in the general group, conversely, there was a tendency to its decrease to borderline values (75,95±10,35%) ($p > 0,05$). At the same time, concentration of fibrinogen in those patients was considerably increased, twice exceeding the control values ($p < 0,001$).

Mean values of anticoagulant protein C activity, compared with the control group, were significantly decreased to lower limit of normal (80,05±1,47% versus 100±20%) ($p < 0,05$).

According to the level of soluble fibrin, the patients were divided into three groups: group 1 -up to 3,0 μg/ml (n=28); group 2 – 3,1-3,9 μg/ml (n=38); group 3 – 4,0 μg/ml and over (n=22).

Taking into consideration, that soluble fibrin can serve as pre-thrombosis indicator, the decision was made to study other indices of hemostasis in the groups, formed according to soluble fibrin concentration (Table II).

Evaluation of soluble fibrin concentrations in three groups, as well as their comparison with general group, demonstrated that only 28 patients (31,8%) of group 1 had normal fibrin value. The values of soluble fibrin level in group 2 (38 patients – 43,2%) were significantly different

as compared to group 1 (p <0,001) and the control group (p <0,02); in group 3 (22 patients – 25,0%) they twice exceeded the normal figures (p <0,001) (Table II), being indicative of increased risk of thrombosis in the patients of those groups. The response of other components of hemostasis was evaluated as well. D-dimer value, which by its nature, should to be representative of post thrombosis process, significantly increased from group1 to group 3 (p <0,05÷0,01), but its increase was insignificant as compared to the general group and the control group (p >0,05). It should be noted that the females of group 3 had more adequate response of D-dimer to the increase of soluble fibrin concentration compared to the males, and D-dimer values were 166,67±39,76 ng/ml versus 77,00±10,42 ng/ml, respectively (p <0,05). However, slight increase of D-dimer on the background of considerable increase in soluble fibrin concentration is regarded as insufficient fibrinolysis, again suggesting an increased risk of thrombosis in that group of patients. As fibrinogen is the only enzyme - the source of fibrin, which characterizes general potential of hemostatic clotting system, its concentrations should be representative of concentrations of soluble fibrin and be comparable with them. This statement was confirmed in the studied patients, as evidenced by generally high fibrinogen concentrations in all three groups. Serum fibrinogen level was considerably increased in 62% of patients. At the same time, in group 1 high fibrinogen concentration was observed along with normal soluble fibrin values, and the tendency to slight decrease of its level was seen in group 3, being attributable to "consumption" reaction in formation of high soluble fibrin concentrations. It should be emphasized that the values of anticoagulant protein C do not correspond to such high potential risk of thrombosis. Table 2 shows similar low levels of protein C in all the groups, and in 34% of patients it was lower the borderline values, so, the absence of proportional anticoagulant reaction in dialysis patients to their procoagulant state can be recorded.

Correlation relationships between soluble fibrin and D-dimer, fibrinogen and C protein in the general group were assessed, and the following data were obtained: medium direct relationship between soluble fibrin and D-dimer, (r = 0,56) and the absence of correlation with fibrinogen (r = 0,04) and protein C (r = -0,08).

Those data indicate that in response to accumulation of soluble fibrin, moderate reaction of D-dimer develops, its adequacy requiring further study, and the reaction of anticoagulant component of hemostasis is totally absent. In this case there are minimal changes in fibrinogen concentrations, but they remain high as compared to the control. Discrete evaluation of correlation relationships between the indicated hemostasis factors in the study groups revealed significant differences depending on soluble fibrin concentration. By contrast to the general group, the patients with normal soluble fibrin level there was its high direct correlation relationship with D-dimer (r = 0,70), moderate direct – with fibrinogen(r = 0,56) and moderate negative – with protein C (r = -0,47). Therefore, despite low concentration of soluble fibrin, an adequate process of fibrinolysis does occur, indicated by D-dimer values, but no stimulation of anticoagulant system takes place, and protein C level remains low.

In group 2 (soluble fibrin level 3,1-3,9 μg/ml), correlation coefficient of soluble fibrin and D-dimer sharply decreases to weak (r = 0,11). Such abrupt fall of D-dimer is indicative of exhausted fibrinolytic processes, which go behind the process of accumulation of soluble fibrin from fibrinogen. Similar to the general group, no relationship with protein C (r = -0,07) and fibrinogen was found, being indicative of considerable imbalance between coagulation and anticoagulation systems and fibrinolysis.

Group 3 is characterized by some reactivation of fibrinolysis in response to continuous increase of soluble fibrin, indicated by moderate correlation with D-dimer (r = 0,55), weak correlation with fibrinogen (r = 0,24), but with no adequate response of anticoagulant system and no correlation with protein C (r = -0,04).

Activation of coagulation system in studied patients is evidenced by relative decrease of serum protein C activity on the background of fibrinogen level increase, it being dependent on soluble fibrin contents. Along with the increase of fibrinogen and soluble fibrin concentration, there is no significant change in protein C activity (Fig. 1), but the relationship between protein C and fibrinogen changes in favor of the latter.

Thus, it can be stated that the patients with stage VD CKD treated by hemodialysis have significant inhibition of both fibrinolysis process and anticoagulant properties of blood plasma, on the background of systemic increase of soluble fibrin level and fibrinogenemia.

DISCUSSION

In view of the fact that the level of soluble fibrin (sF) is an early prognostic indicator of coagulation system activation, and simultaneous estimation of soluble fibrin level and D-dimer level can detect balance or imbalance between accumulation and destruction of soluble fibrin [5], the equilibrium between coagulation system and fibrinolysis proves D-dimer level to be equal to the level of soluble fibrin; in case of violation of the equilibrium between those systems and prevalence of the processes of fibrin formation and stabilization over its destruction, D-dimer concentration can remain within normal limits, while sF level can be increased [5], as confirmed by the study.

Two thirds of the patients with stage V CKD, treated by program hemodialysis, were found to have high soluble fibrin concentration, being indicative of coagulation system activation and possible risk of thrombotic event development, and this had been confirmed by recent published studies [5]. Imbalance between sF and D-dimer in study group of patients implies inadequate fibrinolysis.

Glycoprotein-fibrinogen, known as one of the major components of acute phase of hypercoagulation [6], was significantly higher than control estimates in all study groups, maintaining persistant fibrinogenemia. Epide-

miological studies, however, have demonstrated the relationship between serum fibrinogen concentration and thrombogenesis [6], which can be explained by its ability to influence exponentially the blood viscosity.

Thus, procoagulatory process in the patients, treated by program hemodialysis, has the so-called "acute resonance" in the form of increased sF level, inadequate fibrinolysis and fibrinogenemia.

In this respect, anticoagulant system, represented by C protein, is of great significance. Besides, C protein participates in regulation of fibrinolysis process. This study is the first to establish an exhausted state of patient's coagulation system, as decrease of C protein level to lower limits occurred in all the groups, but it didn't interfere with thrombin formation and fibrinolysis activation in crisis. It means that the patients, treated by program hemodialysis, are in constant danger of thrombophylic (iatrogenic) state development. The suggested markers of thrombogenesis make it possible to identify the risk group among those patients, treated by program hemodialysis, by means of available biochemical investigations.

CONCLUSIONS

1. Soluble fibrin is well known to be the marker of "pre-thrombosis", but its level should be evaluated along with D-dimer level, the marker of "post-thrombosis", considerably increasing their cooperative value as thrombogenesis predictors. Besides, D-dimer is an indirect marker of fibrinolysis, and if the latter is inadequate as related to thrombogenesis, D-dimer exhibits no increase correlatively with soluble fibrin level. The majority of patients (68,2%) with stage VD CKD, treated by long term hemodialysis, were found to have significant increase of soluble fibrin level ($p < 0,02$) along with decreased D-dimer to borderline values.
2. C protein, natural anticoagulation inhibitor, proved to be of great importance. In general group of patients there was significant decrease of protein C ($p < 0,05$) on the background of considerable increase of fibrinogen concentration in the majority of patients (62%) ($p < 0,001$).
3. Gender differences in response of patients to hemodialysis in decreased soluble fibrin concentration should be considered. The females were found to have significant increase of D-dimer level ($p < 0,05$) along with the increase of soluble fibrin concentration.
4. Comprehensive estimation of activators and inhibitors of thrombogenesis can serve an indicator of thrombophilias in the patients with stage VD CKD, treated by program hemodialysis.

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