EXPERIMENTAL WORKS

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CHARACTERIZATION OF THE BLOOD COAGULATION SYSTEM IN MORBIDLY OBESE PATIENTS

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Obesity is a complex metabolic disorder that can be followed by blood coagulation disorders, atherosclerosis and atherothrombosis. In the present work, the levels of fibrinogen, soluble fibrin, D-dimer as well as protein C were measured in the blood plasma of 24 morbidly obese patients (the body mass index exceeds 40 kg/m^2) to evaluate the risk of prothrombotic state. The study showed that near by 80% of patients had substantially increased fibrinogen concentration, 33% had increased concentration of soluble fibrin, 42% had increased level of D-dimer in blood plasma as compared to control. According to the results of individual analysis, the high level of fibrinogen and soluble fibrin while reduced protein C indicated the threat of thrombosis, which requires complex diagnostics to be identified. Therefore, simultaneous quantification of hemostatic system biomarkers in the blood plasma is the confident way to predict the risk of thrombotic complications in morbidly obese patients.

K e y w o r d s: hemostasis, obesity, D-dimer, soluble fibrin, protein C, thrombosis.

Obesity, following smoking and war, is the third cause of serious social problems caused by humans. It is now estimated that around 2.1 billion people worldwide (30% of the world's population) are overweight or obese, which is associated with reduced life expectancy, 2.8 million people die every year because of obesity or its complications [1, 2]. In Ukraine, 53.5% of the adult population (>20 years old) are overweight and 21.3% are obese. The proportion of obese men and women is 15.9 and 25.7%, respectively [3].

Severe obesity is a complex metabolic disorder that can lead to atherosclerosis and atherothrombosis. The study of the characteristics of the body of patients with obesity, especially with morbid obesity, occupies a special niche: it is well known the association of excess body weight with type 2 diabetes or insulin resistance, arterial hypertension and dyslipidemia which raises the risk of cardiovascular diseases, such as ischemic heart disease and ischemic stroke [5-7].

In addition, accumulating evidence suggests an association between obesity, metabolic syndrome, and venous thromboembolism [8].

A number of researchers have shown that the relative risk for the development of pulmonary and venous thromboembolism, deep vein thrombosis in patients with obesity, especially with morbid obesity, is two times higher than in patients without it [9, 10].

Hemostasis disorders in morbid obesity are manifested in an increase in the content/activity of coagulation factors (hypercoagulation), increased platelet activity, and activation of endothelial cells due to tissue hypoxia [1, 11, 12]. Risk factors for thrombosis in obese patients include inflammation, increased thrombin formation, and hyperfibrinogenemia. In addition, patients with morbid obesity show hypofibrinolysis due to increased

levels of plasminogen activator inhibitor 1 (PAI-1) [13-15].

Since obesity by itself is a risk factor for the development of venous thromboembolism [4, 16], and the combination of metabolic and hemostatic changes lead to impaired coagulation and fibrinolytic processes, as well as anticoagulant mechanisms, clinical markers of the prothrombotic state may indicate the risk of developing complications of the metabolic syndrome. The aim of the present work was the selection of basic parameters of the coagulation system that are essential for laboratory testing in the blood plasma of morbidly obese patients.

Materials and Methods

Patients. A total of 24 patients with morbid obesity were included in the study. Patients were treated at Shalimov National Institute of Surgery and Transplantation National Academy of Medical Sciences of Ukraine, Kyiv. The mean age of the patients was 46 ± 4 years. The examined group consisted of 19 females and 5 males. The mean body mass index (BMI) was 44 ± 6 kg/m^2. 4 patients were smokers. In all 24 sick people, arterial hypertension appeared, 19 patients (79%) had type 2 diabetes and 5 (21%) had glucose intolerance. 8 (33%) patients suffered from manifestations of deep vein thrombosis (DVT). The control group consisted of 10 healthy volunteers without obesity BMI values (22 ± 3 kg/m^2) with a mean age of 42 ± 3 years.

Ethical approval. Patients signed informed consent prior to blood sampling according to the Helsinki declaration. This study was approved by the Ethics Committee of Shalimov's National Institute of Surgery and Transplantation National Academy of Medical Sciences of Ukraine, Kyiv (02.08.2015, N4).

Materials. Human blood plasma, immunodiagnostic test-systems (Palladin Institute of biochemistry, Kyiv, Ukraine), thrombin-like enzyme from snake venom (Palladin Institute of biochemistry, Kyiv, Ukraine), protein C-specific chromogenic substrate S2236 (p-Glu-Pro-Arg-pNa).

Methods. Fibrinogen. Fibrinogen concentration in the blood plasma was determined by the modified spectrophotometric method. Blood plasma (0.2 ml) and PBS (1.7 ml) were mixed in a glass tube. Coagulation was initiated by the addition of 0.1 ml of thrombin-like enzyme from the venom of *Aghistrodon halys halys* (1 NIH/ml) to prevent fibrin cross-linking. The mixture was incubated for 30 min at 37°C. The fibrin clot was removed and resolved in 5 ml of 1.5% acetic acid. The concentration of protein was measured using spectrophotometer POP (Optizen, Korea) at 280 nm (ε = 1.5) [17].

Soluble fibrin. Soluble fibrin was detected using sandwich ELISA with monoclonal antibodies produced at Palladin Institute of biochemistry of NAS of Ukraine. Fibrin-specific monoclonal antibody I-3C was used as catch-antibody. Biotinilated monoclonal antibody II-4d that has epitope at the NH_2-terminal fragment of γ-chain of D-region of fibrinogen molecule was used as a tag-antibody optical density was measured at 492 nm using multiplate reader RT 2100C (Rayto, China). [18].

D-dimer. D-dimer was detected using sandwich ELISA as described above for soluble fibrin with modification. Biotinilated DD-specific monoclonal antibody III-3B that has epitope at the NH_2-terminal fragment of Bβ-chain of D-region of fibrin(ogen) produced at Palladin Institute of biochemistry of NAS of Ukraine was used as the catch-antibody [18].

Protein C. Total protein C level in blood plasma was determined using protein C activator and protein C-specific chromogenic substrate S2236 (p-Glu-Pro-Arg-pNa) [19]. In a well of 96-well plate 0.02 ml of studied blood plasma sample, 0.03 ml of S2236 solution (0.25 mM) and 0.03 ml of protein C activator solution were admixed in the TBS with 0.001 M $CaCl_2$ at final volume of 0.25 ml. The generation of colorful *p*-nitroaniline (pNa) was monitored at 405 nm using ThermoMultiscan (ThermoFisher, USA). Results were presented as % from control values.

Statistics. Statistical analysis was done using SPSS (statistical package for social science) version 17. Data are expressed as mean ± SD to describe quantitative variables and number of percentage to describe qualitative variables. The Student's *t*-test was used to assess the difference between two study groups (obese and control) for fibrinogen, D-dimer, soluble fibrin and protein C. $P < 0.05$ was considered significant.

Results

Characterization of cohort. Fibrinogen. Among the study group, only four people (20%) had fibrinogen levels in line with the norm. The rest 20 patients (approximately 80 %) had substantially increased fibrinogen concentration (5.6 ± 1.8 against 2.5 ± 0.5 mg/ml in control) (Fig. 1). Statistical analysis of the Student's *t*-test showed a significant difference between obese and control groups with $P = 0.005$. In most cases hyperfibrinogenemia

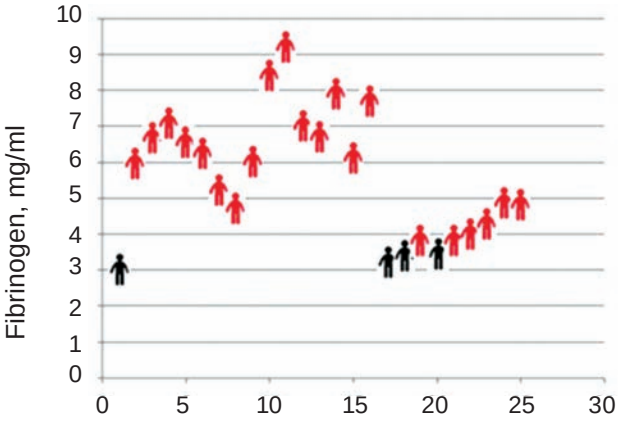

Fig.1. Distribution of morbidly obese patients according to the concentration of fibrinogen (marked in red are patients whose numbers are significantly different from control)

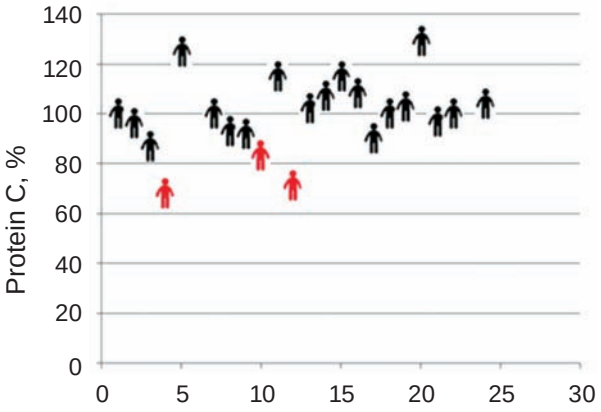

Fig.2. Distribution of morbidly obese patients according to the level of protein C (marked in red are patients whose numbers are significantly different from control)

is considered as an evidences of chronic low-grade inflammation and increased risk of intravascular clotting.

Protein C. Assessment of the state of the hemostasis system in patients with morbid obesity did not reveal significant changes in the anticoagulant level: we did not find any significant decrease in the level of protein C which is the main anticoagulant factor (Fig. 2).

D-dimer. Approximately 42 % of patients (10 out of 24) had the level of D-dimer increased twice or more compared to the control (6 patients had 1.5-2.0 times higher, and 6 had 3 and more times higher against 100±50 ng/ml for developed test) (Fig. 3). However statistical calculation did not show a significant difference between study groups with $P = 0.06$.

Soluble fibrin. Also, there was not a significant difference in the amount of soluble fibrin in the plasma of obese patients compared to the healthy group ($P = 0.125$). About 33% of patients had increased concentration of SF (9 out of 24) (Fig. 4).

Personalized analysis. For individual analysis, we chose patients with a high level of SF in the blood plasma and with a level that corresponds to the norm, since the degree of activation of the blood coagulation system correlates with the accumulation of this very marker of thrombophilia. Table shows

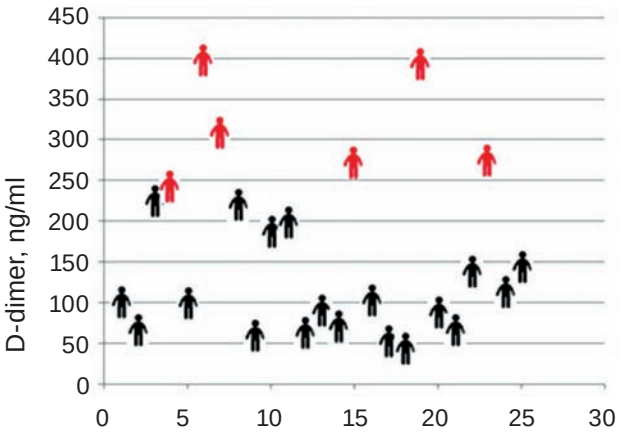

Fig. 3. Distribution of morbidly obese patients according to the concentration of D-dimer (marked in red are patients whose numbers are significantly different from control)

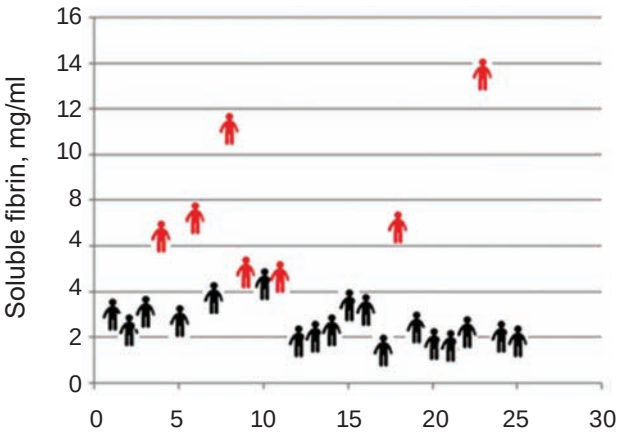

Fig. 4. Distribution of morbidly obese patients according to the concentration of soluble fibrin (marked in red are patients whose numbers are significantly different from control)

Ta b l e. Individual blood plasma samples from patients with morbid obesity, with activated blood coagulation system

Parameters	Patients					
	A	B	C	D	E	Control (donors)
Fibrinogen, g/l	7.0 ± 0.6	4.3 ± 0.2	3.4 ± 0.3	7.1 ± 0.4	3.0 ± 0.2	3.0 ± 0.5
Soluble, fibrin mg/l	1.8 ± 0.2	13.5 ± 0.7	6.8 ± 0.5	7.7 ± 0.6	5.2 ± 0.6	3.0 ± 0.3
D-dimer, ng/l	62 ± 29	274 ± 63	42 ± 8	250 ± 58	593 ± 77	100 ± 50
Protein C, %	71 ± 8	65 ± 3	100 ± 25	68 ± 9	96 ± 17	100 ± 20

the results of the analysis of individual blood plasma samples from patients with morbid obesity, which should be taken into account when monitoring the condition of patients.

Patient A. Patient's blood coagulation system is not activated SF and D-dimer are almost absent. However, high content of fibrinogen and a significant consumption of protein C indicate the development of an inflammatory process and the appearance of thrombin in the bloodstream, respectively. It is necessary to pay attention to the level of PC: if the indicator does not recover during the course of appropriate therapy, it is advisable to conduct genetic studies.

Patient B. The level of D-dimer and soluble fibrin increased by 4 times relative to the normal and a very low level of protein C indicates a high degree of activation of the patient's blood coagulation system. Anticoagulant therapy is required.

Patient C. Practically absent D-dimer and 2.2-fold increased SF level indicates an imbalance between pro- and anticoagulants. Despite a slight degree of activation, the patient is at risk, since any inflammatory process or surgical intervention can cause further development of thrombophilia.

Patient D. A 3-fold increase in the level of D-dimer, a 2.5-fold increase in SF and a decrease in the content of PC indicate the activation of the blood coagulation system, which is especially dangerous against the background of a high level of fibrinogen. The patient requires anticoagulant therapy because he is at risk of developing thrombosis.

Patient E. Test results show slight activation of the coagulation system. The high content of D-dimer and SF indicates that the coagulation and fibrinolytic processes are in dynamic equilibrium. However, despite the apparent well-being, the presence of RF indicates the need to monitor the patient's condition.

Discussion

Evaluation of indicators of the state of hemostatic system could be perspective for determining the coagulation potential of patients with morbid obesity. Despite the presence of a large amount of accumulated data that shed light on the pathogenesis of thrombosis in morbid obesity, the strategy for preventing thrombosis in such patients needs to be improved. The most informative markers of activation of the coagulation process are required to help identify patients at increased risk of thrombosis. Our study focused on determining the content of fibrinogen, soluble fibrin, D-dimer and protein C – indicators that most accurately reflect the balance between the procoagulant and fibrinolytic links of the hemostasis system in the case of morbid obesity.

Obesity is characterized by hyperfibrinogenemia, which, by changing the rheological properties of blood and clot structure, impairs blood flow in the microcirculation. Fibrinogen promotes arterial and venous thrombosis by increasing fibrin formation and could trigger atherosclerosis through vascular smooth muscle and endothelial cell proliferation [10]. In our work, 80% of patients with morbid obesity showed a significant relative to the norm increase in the content of fibrinogen (4-9 g/l versus 3.0 g/l) which may also indicate the development of an inflammatory process. Since hyperfibrinogenemia is an independent factor in the development of thrombosis, high fibrinogen concentration in the blood plasma of morbidly obese patients is compelling evidence of the risk of hypercoagulability in such patients. Our results are consistent with literature data [20-23] and indicate the activation of the coagulation system, which leads to an increase in the formation of thrombin, a marker of hypercoagulability. Regular laboratory testing of the basic parameters of the

blood coagulation system could be recommended to morbidly obese patients due to increased fibrinogen level in their blood.

Changes in the coagulation process in obese individuals, especially those with morbid obesity, are complex. On the one hand, they include an increase in the content/activity of blood coagulation factors, hyperfibrinogenemia and excessive platelet activity. On the other hand, in obese individuals, fibrinolysis is inhibited. A direct relationship has been established between morbid obesity and an increased level of tissue factor, blood coagulation factors VII and VIII, von Willebrand factor, antithrombin III, TAT complex and fibrinogen and, indirectly, endothelial damage as an independent factor contributing to the risk of thrombosis [24-29].

Cleavage of fibrin during fibrinolysis leads to the formation of D-dimer. The concentration of D-dimer in the blood is proportional to the activity of fibrinolysis and the amount of lysed fibrin. D-dimer is a marker of activation of intra- and extravascular coagulation and fibrinolysis. In our study, elevated levels of D-dimer (250 ng/ml relative to 75-100 ng/ml) were found in 42% of patients with morbid obesity. A high level of soluble fibrin (≥4 mg/l relative to 3 mg/l), a marker of activation of intravascular coagulation, was detected in 33% of patients (8 of 24). As for the level of protein C, there were no significant changes in the content/activity of this physiological anticoagulant: only 12% of patients (3 out of 24) showed low levels (68-71% relative to 100%). For individual analysis, we chose patients with a high level of SF in the blood plasma and with a level that corresponds to the norm, since the degree of activation of the blood coagulation system correlates with the accumulation of this very marker of thrombophilia (Fig. 4).

From the results of an individual analysis (Table), it follows that with a high level of fibrinogen and soluble fibrin and reduced protein C at the same time there is a real threat of thrombosis, which requires complex diagnostics to be identified. The methods of laboratory diagnostics we have chosen confirm the need for their integrated use.

However, according to an individual analysis, low level of protein C and accumulation of SF was observed in the samples with a normal range of D-dimer which indicates pathological thrombin generation.

On the other hand, a high level of D-dimer (more than 250 ng/ml) in most cases was accompanied by accumulation of SF. As far as SF is the product of thrombin generation and fibrin production while D-dimer is the result of fibrinolysis of stabilized fibrin, one can conclude that these patients have well-balanced hemostasis.

Therefore, characterization of different biomarkers that could be used to identify patients with increased risk of thrombosis in the perioperative setting of surgery as well as in the long run needs to be established.

Conclusions. More than 30% of patients with normal levels of D-dimer (100-200 ng/ml) had a high risk of thrombosis according to high levels of fibrinogen and SF. Two of patients also additionally had decreased level of protein C that was assumed as the treat of intravascular thrombus formation. Simultaneous quantification of SF, D-dimer and protein C is the only confident way to predict the risk of thrombotic complications in morbidly obese patients.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ХАРАКТЕРИСТИКА СИСТЕМИ ЗГОРТАННЯ КРОВІ У ПАЦІЄНТІВ ІЗ ПАТОЛОГІЧНИМ ОЖИРІННЯМ

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Ожиріння – це складне порушення обміну речовин, яке супроводжується порушенням згортання крові, атеросклерозом і атеротром-

бозом. У даній роботі було визначено рівень фібриногену, розчинного фібрину, D-димеру, а також протеїну С у плазмі крові 24 пацієнтів із патологічним ожирінням (індекс маси тіла вище 40 кг/м^2) для оцінки ризику протромботичного стану. Дослідження показало, що у біля 80% хворих суттєво підвищилася концентрація фібриногену, у 33% – розчинного фібрину, у 4 % – рівень D-димеру в плазмі крові порівняно з контролем. За результатами індивідуального аналізу високий рівень фібриногену та розчинного фібрину за зниженого протеїну С вказує на загрозу тромбозу, що потребує комплексної діагностики. Таким чином, одночасне кількісне визначення біомаркерів системи гемостазу в плазмі крові є впевненим способом прогнозування ризику тромботичних ускладнень у пацієнтів із патологічним ожирінням.

Ключові слова: гемостаз, ожиріння, D-димер, розчинний фібрин, протеїн С, тромбоз.

References

1. King RJ, Ajjan RA. Vascular risk in obesity: Facts, misconceptions and the unknown. *Diab Vasc Dis Res.* 2017; 14(1): 2-13.
2. Gregg EW, Shaw JE. Global Health Effects of Overweight and Obesity. *N Engl J Med*. 2017; 377(1): 80-81.
3. Clinical Research Uuraine. Professional portal Available online: https://crupp.org/obesity/
4. Cominato L, Franco RR, Ybarra M, Frascino AV, Steinmetz L, Ferraro AA, Aivazoglou Carneiro JD, Damiani D. Obesity as a Thrombogenic and Cardiovascular Risk Factor in Children. *Horm Res Paediatr.* 2021; 94(11-12): 410-415.
5. Grundy SM. Metabolic syndrome: connecting and reconciling cardiovascular and diabetes worlds. *J Am Coll Cardiol*. 2006; 47(6): 1093-1100.
6. Lind L, Siegbahn A, Ingelsson E, Sundström J, Arnlöv J. A detailed cardiovascular characterization of obesity without the metabolic syndrome. *Arterioscler Thromb Vasc Biol*. 2011; 31(8): e27-34.
7. Morange PE, Alessi MC. Thrombosis in central obesity and metabolic syndrome: mechanisms and epidemiology. *Thromb Haemost.* 2013; 110(4): 669-680.
8. Blokhin IO, Lentz SR. Mechanisms of thrombosis in obesity. *Curr Opin Hematol*. 2013; 20(5): 437-444.
9. Darvall KAL, Sam RC, Silverman SH, Bradbury AW, Adam DJ. Obesity and thrombosis. *Eur J Vasc Endovasc Surg.* 2007; 33(2): 223-233.
10. Craft MK, Reed MJ. Venous thromboembolic disease and hematologic considerations in obesity. *Crit Care Clin*. 2010; 26(4): 637-640.
11. Badimon L, Hernández Vera R, Vilahur G. Atherothrombotic risk in obesity. *Hamostaseologie*. 2013; 33(4): 259-268.
12. Samad F, Ruf W. Inflammation, obesity, and thrombosis. *Blood*. 2013; 122(20): 3415-3422.
13. Świątkowska-Stodulska R, Kazimierska E, Sworczak K, Częstochowska E. Hemostatic disturbances in obesity. *Wiad Lek*. 2007; 60(3-4): 185-188.
14. Michalska M, Iwan-Ziętek I, Gniłka W, Dąbrowiecki S, Góralczyk B, Góralczyk K, Drela E, Rość D. PAI-1 and α2-AP in patients with morbid obesity. *Adv Clin Exp Med*. 2013; 22(6): 801-807.
15. Campello E, Zabeo E, Radu CM, Spiezia L, Gavasso S, Fadin M, Woodhams B, Vettor R, Simioni P. Hypercoagulability in overweight and obese subjects who are asymptomatic for thrombotic events. *Thromb Haemost.* 2015; 113(1): 85-96.
16. Yuan S, Bruzelius M, Xiong Y, Håkansson N, Åkesson A, Larsson SC. Overall and abdominal obesity in relation to venous thromboembolism. *J Thromb Haemost.* 2021; 19(2): 460-469.
17. Sokolovska AS, Chernyshenko TM, Ivanenko TI. Comparative characteristic of fibrinogen level determination methods in blood plasma. *Exper Clin Phisiol Biochem*. 2002; (3): 82-86. (In Ukrainian).
18. Nikulnikov PI, Liksunov OV, Ratushnyuk AV, Lugovskoy EV, Kolesnikova IM, Lytvynova LM, Kostyuchenko OP, Chernyshenko TM, Gornytska OV, Platonova TM. Estimation of state of the blood coagulation system after the operation for abdominal aorta aneurysm. *Klin Khirurg.* 2012; (9): 32-36.
19. Roshan TM, Stein N, Jiang XY. Comparison of clot-based and chromogenic assay for the determination of protein c activity. *Blood Coagul Fibrinolysis.* 2019; 30(4): 156-160.

20. Solá E, Vayá A, Simó M, Hernández-Mijares A, Morillas C, España F, Estellés A, Corella D. Fibrinogen, plasma viscosity and blood viscosity in obesity. Relationship with insulin resistance. *Clin Hemorheol Microcirc*. 2007; 37(4): 309-318.
21. Ay L, Kopp HP, Brix JM, Ay C, Quehenberger P, Schernthaner GH, Pabinger I, Schernthaner G. Thrombin generation in morbid obesity: significant reduction after weight loss. *J Thromb Haemost*. 2010; 8(4): 759-765.
22. Kopp CW, Kopp HP, Steiner S, Kriwanek S, Krzyzanowska K, Bartok A, Roka R, Minar E, Schernthaner G. Weight loss reduces tissue factor in morbidly obese patients. *Obes Res*. 2003; 11(8): 950-956.
23. Jabber HA, Al-Rubaie HA, Alalsaidissa J. The relationship between obesity and plasma level of factor V and fibrinogen. *J Fac Med Baghdad*. 2017; 59(1): 53-59.
24. Firszt-Adamczyk A, Ruszkowska-Ciastek B, Adamczyk P, Szafkowski R, Firszt M, Ponikowska I, Moe K, Iwan-Ziętek I, Góralczyk B, Góralczyk K, Rość D. Effect of a 3-Week Low-Calorie Diet and Balneological Treatment on Selected Coagulation Parameters in Morbidly Obese Patients. *Adv Clin Exp Med*. 2016; 25(4): 755-761.
25. De Pergola G, Pannacciulli N. Coagulation and fibrinolysis abnormalities in obesity. *J Endocrinol Invest*. 2002; 25(10): 899-904.
26. Bladbjerg EM, Stolberg CR, Juhl CB. Effects of Obesity Surgery on Blood Coagulation and Fibrinolysis: A Literature Review. *Thromb Haemost*. 2020; 120(4): 579-591.
27. Grover SP, Mackman N. Tissue Factor: An Essential Mediator of Hemostasis and Trigger of Thrombosis. *Arterioscler Thromb Vasc Biol*. 2018; 38(4): 709-725.
28. Samuels J, Lawson PJ, Morton AP, Moore HB, Hansen KC, Sauaia A, Schoen JA. Prospective assessment of fibrinolysis in morbid obesity: tissue plasminogen activator resistance improves after bariatric surgery. *Surg Obes Relat Dis*. 2019; 15(7): 1153-1159.
29. Singh A, Foster GD, Gunawardana J, McCoy TA, Nguyen T, Vander Veur S, Komaroff E, Rao AK. Elevated circulating tissue factor procoagulant activity, factor VII, and plasminogen activator inhibitor-1 in childhood obesity: evidence of a procoagulant state. *Br J Haematol*. 2012; 158(4): 523-527.