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State of plasma hemostasis in patients with hypertension in combination with non-alcoholic fatty liver disease

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Abstract

Background. Among liver diseases, non-alcoholic fatty liver disease (NAFLD) is the most common. NAFLD is an independent risk factor for the development and progression of cardiovascular diseases. **Objective.** To determine the state of the anticoagulant, fibrinolytic and coagulation hemostasis in patients with hypertension and concomitant non-alcoholic fatty liver disease in the presence of obesity. **Materials and methods.** 150 patients (64 men and 86 women) were examined. Groups of patients: I - 50 patients with HT stage 2, II - 48 patients with NAFLD without HT, group III - 52 patients who had HT stage 2 with concomitant NAFLD. **Results.** PT value decrease in the NAFLD group by 12.2% ($p<0.01$). The level of TT is reduced by 16.2% ($p<0.001$) in the group with NAFLD. APTT decreases in patients with NAFLD by 13.3% ($p<0.001$) and when combined with NAFLD with hypertension by 13.6% ($p<0.05$). Fibrinogen increases in the HT group by 31.5% ($p<0.001$), and in the NAFLD group combined with HT - by 39.8% ($p<0.001$). SFMK levels significantly increase in all groups of patients: in patients with hypertension - 4.9 times ($p<0.001$), with NAFLD - 3

times (p<0.001), in the NAFLD+HT group - 5.3 times (p<0.001). There is a decrease in AT III by 16.4% in both the HT group (p<0.01) and the NAFLD group (p<0.01), combined pathology leads to more significant inhibition AT III - by 20.3% (p<0.001). HDF increases in the HT group - by 47% (p<0.001), in the NAFLD group - by 78% (p<0.001), in the NAFLD + HT group - 2.4 times (p<0.001).). **Conclusions.** In hypertension combined with NAFLD, depletion of anticoagulant and fibrinolytic potential against the background of activation of coagulant hemostasis, indicate the presence of prothrombogenic changes.

Keywords: non-alcoholic fatty liver disease, hemostasis, hypertension, anticoagulant, fibrinolysis, coagulation.

Background

Increased blood pressure (BP) is the largest factor in global morbidity and mortality worldwide, as it contributes to the development of ischemic and hemorrhagic damage to target organs. The magnitude of blood pressure has a positive and continuous correlation with the risk of developing strokes and coronary heart disease, significantly affecting the overall mortality (Poulter, Prabhakaran, & Caulfield, 2015).

Although there are differences in the average BP in different countries, there are no global trends in the average blood pressure levels over the past decades. (Mancia et al., 2013). Excessive weight gain, especially visceral obesity, is the main cause of hypertension, and ranges from 65% to 75% of the risk of primary hypertension in humans (Hall, do Carmo, da Silva, Wang, & Hall, 2015).

Obesity is one of the main modifiable risk factors for the occurrence of the pathology of the cardiovascular system, causing its rapid progression, more severe course and a high frequency of complications.

In addition, obesity is the most significant factor associated with non-alcoholic fatty liver disease (NAFLD). According to numerous studies, an increase in body mass index (BMI) is an independent predictor of the formation of fatty liver infiltration (Rinella, 2015).

Although NAFLD is strongly associated with obesity, insulin resistance, and type 2 diabetes, many of NAFLD are not obese, and many people with NAFLD do not have type 2 diabetes (Byrne & Targher, 2015).

Currently, there are a lot of studies that confirm the relationship between hypertension (HT) and NAFLD. It is proved that the presence of hypertension increases or provokes the development of NASH. Thus, in patients with hypertension in more than half of cases, NAFLD is also found without other risk factors for the development of liver diseases. The

greatest number of cases of NAFLD (≈80%) is diagnosed in the non-dippers group - a person with insufficient nighttime blood pressure.

But despite the frequent combined course of hypertension and NAFLD, according to modern concepts, NAFLD is positioned as an independent risk factor for the development and progression of cardiovascular disease (CVD). Given the clinical and social significance of CVD, the problem of early diagnosis and treatment of NAFLD requires the coordination of efforts of doctors of all specialties. The accumulated clinical experience of studying hypertension, as the most common disease among CVDs, allows us to regard high blood pressure as one of the etiological factors of thrombogenic changes in the blood. The relationship between hemostatic changes and blood pressure was confirmed by the results of many studies more than 20 years ago. It is proved that the fibrinolytic potential has a negative correlation with systolic blood pressure (Junker, Heinrich, Schulte, Erren, & Assmann, 1998). In patients, the activity of coagulation factors increases, the level of coagulation inhibitors (antithrombin III, protein C, protein S) decreases and fibrinolysis is slowed down. (Junker et al., 1998; Lip, Blann, Jones, Lip, & Beevers, 1997; Makris et al., 1997; Woodward et al., 1997). In turn, NAFLD is also accompanied by procoagulogenous changes in hemostasis. Tripodi A. et al. Demonstrated the presence of thrombophilic changes in the blood of patients with NAFLD, thrombin, factor VIII and protein C were the object of their research (Tripodi et al., 2014). Lallukka S. et al. Discovered procoagulative imbalance in NAFLD due to increased activity of factors IX, XIII and fibrinogen (Lallukka, Orho-Melander, Lundbom, Olkkonen, & Yki-Järvinen, 2016). Despite the achieved understanding of the general pathogenic mechanisms of development of NAFLD and hypertension, this comorbid pathology remains the subject of numerous discussions and studies, evoking the interest of doctors of various specialties. But the direct study of fibrinolytic factors, anticoagulant and coagulation units of blood coagulation in patients with combined course of NAFLD and hypertension was not conducted.

Objective

To determine the state of the anticoagulant, fibrinolytic and coagulation hemostasis in patients with hypertension and concomitant non-alcoholic fatty liver disease in the presence of obesity.

Materials and methods

150 patients (64 men and 86 women) were examined on the basis of the Kyiv Railway Clinical Hospital #2 of branch "Health center" of the Public Joint Stock Company "Ukrainian Railway" in the period from 2015-2018. The average age of patients is 58.7±8.6 years. Three

groups of patients were identified: I - 50 patients with stage II HT, II - 48 patients with NAFLD without HT, group III - 52 patients who had stage II HT with concomitant NAFLD. All patients had I-III degree of obesity. The control group consisted of 15 healthy individuals of comparable age and sex. Patients conducted general clinical trials; for verification of NAFLD - ultrasound examination of the abdominal cavity.

To achieve this goal, anticoagulant, fibrinolytic, and coagulation units of plasma hemostasis were studied using special laboratory tests.

The coagulation activity of the blood was studied using the determination of prothrombin time (PT), international normalization ratio (INR), thrombin time (TT), activated partial thromboplastin time (APTT), fibrinogen and soluble fibrin-monomer complexes (SFMK). The fibrinolytic activity of the blood was studied by determining Hageman (XII-a)-dependent fibrinolysis (HDF) and plasminogen (PG), and the state of the anticoagulant hemostasis by analyzing protein C (PC) and antithrombin III (AT III). Statistical data processing was carried out using the statistical package Portable Statistic 10 StatSoft, Inc., USA. The critical level of significance when testing statistical hypotheses was taken to be 0.05. Non-parametric statistical methods were used for the analysis of anticoagulant and fibrinolytic hemostasis: U-Mann-Whitney test, Kruskal-Wallis test (Kruskal-Wallis H-test), because small sample sizes were used, and the value in the groups did not follow the law of normal distribution.

Results

Comparing groups of patients with the control cohort demonstrate significant differences in the PT value only in the group of patients with NAFLD — a decrease in the level of the indicator by 12.2% ($p<0.01$). In the intergroup analysis, it was found that in the NAFLD group combined with hypertension, the level of PT is lower by 10.8% ($p<0.01$), in the case of isolated NAFLD - by 13.1% ($p<0.001$) compared with the HT group. The value of the standardized indicator of INR has a significant difference compared with the control group only in the NAFLD group - lower by 8.4% ($p<0.01$). The level of TT is reduced by 16.2% ($p<0.001$) in the group of patients with NAFLD compared to control, and has significant differences between the HT group and NAFLD - 12.1% less in patients with NAFLD ($p<0.001$). APTT decreases in patients with NAFLD by 13.3% ($p<0.001$) and when combined with NAFLD with hypertension by 13.6% ($p<0.05$) when compared with the control measurement. From the indicators of the HT group, the value of the APTT of the NAFLD group is significantly different - by 21.8% less ($p<0.001$), and the NAFLD + HT group - lower by 22.1% ($p<0.001$). The amount of fibrinogen increases in the HT group by

31.5% (p<0.001), and in the NAFLD group combined with HT - by 39.8% (p<0.001). The fibrinogen value is lower in patients with NAFLD compared with the HT group by 14.5% (p<0.001), and by 19.6% (p<0.001) compared with the values in the combined pathology. SFMK levels significantly increase in all groups of patients: in patients with hypertension - 4.9 times (p<0.001), with NAFLD - 3 times (p<0.001), in the NAFLD + HT group - 5.3 times (p<0.001). Intergroup comparisons revealed an increase in SFMC in patients with hypertension by 62% (p<0.001), in patients with combined course of hypertension and NAFLD - by 77% (p<0.001) in patients with isolated NAFLD. In the NAFLD + HT group, SFMK level is higher by 9.6% (p<0.05) than in patients with HT (Table 1).

Table 1

Values of plasma hemostasis in different groups of patients

Value	Control	HT (1)	NAFLD (2)	NAFLD+ HT (3)	P(1-2)	P(1-3)	P(2-3)
PT, s	19,3σ=0,67	19,5 σ=1,92	16,95σ=2,93**	17,4σ=3,5	p<0,001	p<0,01	p=0,512
INR	0,83 σ=0,57	0,86 σ=0,09	0,76 σ=0,12**	0,78σ=0,14	p<0,001	p<0,01	p=0,468
TT, s	10,6 σ=0,85	10,1σ=1,58	8,88σ=1,83***	10,3σ=1,21	p<0,001	p=0,767	p<0,001
APTT, s	30,2 σ=3,21	33,5 σ=6,1	26,2σ=2,83***	26,1σ=9,29*	p<0,001	p<0,001	p=0,235
Fibrinogen, g/L	2,89 σ=0,6	3,8σ=0,47***	3,25σ=0,8	4,04σ=0,8***	p<0,001	p=0,089	p<0,001
SFMC 10^{-2} g/L	4,1 σ=0,6	19,9σ=3,75***	12,3 σ=5,2***	21,8σ=4,16***	p<0,001	p<0,05	p<0,001
AT III, %	94,4 (σ=16,8)	78,9 (σ=7,96)**	78,9 (σ=12,9)**	75,2 (σ=11,5)***	p=0,798	p=0,217	p=0,247
PC, %	100 (σ=18,8)	74,2 (σ=10,7)***	90,5 (σ=12,6)*	85,9 (σ=33,2)**	p<0,05	p<0,05	p=0,506
HDF, min	8,7 (σ=2,35)	12,8 (σ=3,06)***	15,5 (σ=5,3)***	20,5 (σ=5,89)***	p<0,05	p<0,001	p<0,001
PG, %	90,6 (σ=7,2)	76,7 (σ=4,01)***	71,7 (σ=17,4)***	75,7 (σ=13,1)***	p=0,322	p=0,812	p=0,283

Notes: 1. PT - prothrombin time; INR - international normalization ratio; TT, thrombin time; APTT - activated partial thromboplastin time; SFMC - soluble fibrin-monomer complexes; AT III - antithrombin III; PC - protein C; HDF - Hageman-dependent fibrinolysis; PG - plasminogen; HT - hypertension; NAFLD - non-alcoholic fatty liver disease 2. * - significance of change according to the Mann-Whitney criterion (U Test) compared with the control group: * – p<0.05; ** – p<0.01; *** – p<0.001.

According to the results of our own studies, when compared with the control group, there is a decrease in AT III by 16.4% in both the HT group (p<0.01) and the NAFLD group (p<0.01), combined pathology leads to more significant inhibition AT III - by 20.3% (p<0.001). Significant differences in PC levels when compared with the control cohort were found in the HT group - the value decreases by 26% (p<0.001), in the NAFLD group - by 9.5% (p<0.05), in the NAFLD + HT group - by 14.1% (p<0.01). According to our studies, the level of PG is significantly reduced with HT - by 15% (p<0.001), with NAFLD - by 20.9%

($p<0.001$), NAFLD + HT - by 16.5% ($p<0.001$) control group, and has no significant differences in intergroup comparison. The definition of HDF is the determination of the time of dissolution of a retraged fibrin clot under the influence of the plasmin proteolytic enzyme. We observe the lengthening of this time in all groups compared with the control: in the HT group - by 47% ($p<0.001$), in the NAFLD group - by 78% ($p<0.001$), in the NAFLD + HT group - 2.4 times ($p<0.001$). Unlike patients with HT, the duration of HDF is 21% longer with NAFLD ($p<0.05$), and 60% with NAFLD + HT ($p<0.001$). Comparison of groups with liver damage demonstrates a longer clot lysis time in the case of a combined pathology - by 32% ($p<0.001$).

Discussion

In the group of patients with AH, there were no significant differences in PT, INR, APTT and TT levels from control indicators, although a tendency for their levels to increase is in accordance with the data described in the literature (Hassan & Merghani, 2016; JiskaniS, Memon, & Naseem, 2017; Nnenna Adaeze, Uchenna Emeribe, Abdullahi Nasiru, Babayo, & Uko, 2014). NAFLD is characterized by a decrease in PT, INR, TT, APTT, which indicates an acceleration of coagulation in this group of patients, and corresponds to the literature data (Fargion, Porzio, & Fracanzani, 2014; Stine, Intagliata, Northup, & Caldwell, 2017). In cases of accession of hypertension to NAFLD, a significant shortening of the time of clot formation is observed by the internal mechanism of activation of coagulant hemostasis. While the value of PCMC significantly exceeds the benchmarks in all groups, with a high level in patients with hypertension, connected with NAFLD. Since SFMC is a marker of thrombosis (Elazab Elged, El-Gamal, Bastawy, & Saeed, 2016), an increase in its level indicates an increase in the prothrombogenic activity of the blood when NAFLD is attached to hypertension. Fibrinogen level rises in response to systemic inflammation, tissue damage and the presence of cancer. It has been established that an increased level of fibrinogen in inflammation, cancer and other diseases is the cause of thrombosis (Davalos & Akassoglou, 2012; Repetto & De Re, 2017). According to the results of our own research, the value of fibrinogen increased in HT patients, as well as the levels of this indicator significantly increase when HT is added to NAFLD, by 19.6% ($p <0.001$) as compared with an isolated course of NAFLD. Since SFMC and fibrinogen characterizes the final link of thrombosis in the blood coagulation cascade, it is possible to consider the comorbid course of hypertension and NAFLD as a factor of prothrombogenic changes in hemostasis.

According to the results of our studies in patients with hypertension and patients with NAFLD suppression of anticoagulant hemostasis observed by reducing levels of AT III and sun, consistent with the data described in the literature [8, 14]. In the case of a combination of HT and NAFLD, the level of AD BP decreases to the same extent as with an isolated course of HT or NAFLD. Decrease in PC is more marked in patients with hypertension than in patients with NAFLD and in patients with comorbid course of these diseases. There is a depletion of anti-bursting potential in all groups of patients, due to a decrease in the activity of protein anticoagulants. According to the results of our own research, a decrease in blood fibrinolytic activity was also found in the HT and NAFLD groups, which corresponds to the literature data. In addition, for the first time it was found that the combination of these diseases leads to a more significant depression of fibrinolysis due to the prolongation of the Hageman-dependent time, and a decrease in the level of plasminogen is the same in all groups of patients.

Determination of coagulation state, anticoagulant and fibrinolytic links of hemostasis do not take into account the entire volume of hemostatic changes occurring during the comorbid course of hypertension and NAFLD. There is a need for a more extensive study of the hemostatic system, including the platelet part of hemostasis in this cohort of patients.

Conclusions

1. In patients with hypertension and patients with NAFLD suppression of anticoagulant hemostasis is observed by reducing the activity of AT III and PC. Reduction of blood fibrinolytic activity in patients with hypertension, NAFLD and their combination manifests itself in lengthening the time of dissolution of the fibrin clot and inhibition of plasminogen. The increase in the time of Hageman-dependent fibrinolysis is more significant in the HT group combined with NAFLD, whereas the level of plasminogen decreases equally in all groups of patients.

2.Patients with NAFLD has accelerating of coagulation at all stages of thrombus formation, while the combination of HT and NAFLD is accompanied by an increase in thrombogenic potential, predominantly at the final stage of blood coagulation.

3. For patients with NAFLD and patients with hypertension associated with NAFLD, activation of the internal coagulation pathway is characteristic, as evidenced by the shortening of the APTT. These changes reflect the presence of thrombophilic changes, creating an additional risk of thrombotic complications in this category of patients.

4. In hypertension connected with NAFLD, depletion of anticoagulant and fibrinolytic potential against the background of activation of coagulant hemostasis, indicate the presence

of prothrombogenic changes, therefore comorbidity of hypertension and NAFLD can be considered a factor of thrombophilic changes of hemostasis.

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