ORIGINAL ARTICLE

Obesity as an Additional Risk Factor for Thrombogenic Changes in Hemostasis in Hypertensive Patients with Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Introduction: Obesity due to the high proportion of visceral adipose tissue is often leads to hypertension (HT), non-alcoholic fatty liver disease (NAFLD). The risk of thrombogenic complications increases in the presence of NAFLD and HT. Considering the occurrence of prothrombotic changes in the blood in patients with NAFLD, HT and obesity, the combination of these diseases may be an additional risk of thrombosis. **Methods:** The research was conducted at the Bogomolets National Medical University, at Department Propaedeutics of Internal Medicine No 1. We examined 152 patients and 15 individuals for control group. **Results:** Mean platelet count was higher in obese patients by 6.4% ($p<0.05$). In patients with NAFLD without HT, platelet count was higher in the presence of obesity by 16% ($p<0.05$), we have seen an increase level of MPV in NAFLD patients with obesity. In the comorbid course of HT and NAFLD, aggregation of platelets stimulated by adrenaline was grown in case of obesity (18%; $p<0.001$) relatively to non-obese patients. In obese patients suffering from HT, these hemostasis changes were characterized by a tendency to accelerate coagulation. There was a reduction in prothrombin time (PT) time by 8.1%, a growth in fibrinogen by 25.8% ($p<0.001$) and soluble fibrin monomeric complexes (SFMC) by 4 times ($p<0.001$) in obesity. **Conclusion:** Obesity increases the thrombogenic activity of blood in both patients with HT and in patients with HT and NAFLD, both due to platelet and coagulation of hemostasis.
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INTRODUCTION

The increase in the level of obesity in the world over the past decades is taking place at a significant pace, creating a global public health crisis. Global estimates in recent years suggest that half a billion people are obese in the world (1).

In the XXI century, obesity is considered as a major non-infectious disease plagued all mankind (2). According to the WHO, in 2016 there were more than 1.9 billion people over the age of 18 who were overweight, of whom 650 million were diagnosed with obesity (3).

Weight gain due to the high proportion of visceral adipose tissue is largely associated with the development of hypertension (HT) and a number of other metabolic changes that lead to development cardiovascular disease (CVD). The relationship between overweight and HT was documented in the Framingham study. The relationship between cardiovascular morbidity and mortality varies depending on the presence of other concomitant cardiovascular predisposing factors. Metabolic changes in patients with increasing blood pressure are more common than in low (4). It is known that in recent years non-alcoholic fatty liver disease (NAFLD) is considered as one of the important metabolic predictors of CVD. NAFLD also increases with incidences of visceral obesity. Increasing frequency of NAFLD is projected unchanged high level of obesity in the world. Today, NAFLD is the one of the biggest reason of long-term liver disease. (5, 6).

There have been conducted a few trials that have shown a relation of weight gain and NAFLD. It has been shown that even a slight increase weight even by 2 kg increases the risk of developing NAFLD. On other hand overall frequency of obesity in patients with fatty liver in the world is 51% (7).

Today, NAFLD is considered as a whole range of diagnoses from steatosis and steatohepatosis to fibrosis. In case it left untreated, steatohepatitis can progress to cirrhosis. NAFLD is one of the main causes of transplantation of liver in developed countries (8). Simultaneously to increasing of number of obese patients, cirrhosis caused by NAFLD is anticipated to be the leading reason of liver transplantation in the next decade; and today NAFLD became the main reason for transplantation of liver in the female population (8).

But main cause of death in patients with NAFLD is CVD, which is mainly due to arterial thrombosis (eg, myocardial infarction, cerebrovascular accident) and venous thrombosis. Hemostatic changes in the blood in NAFLD lead to CVD and their complications (9).

Significant contribution to the development of CVD in NAFLD, which raises the thrombotic threat of by growth of amount of proatherogenic lipids, proinflammatory cytokines, procoagulative and hypofibrinolytic factors (10). Violations of the different levels of hemostasis have thrombogenic abnormalities in paients NAFLD (9). The presence of such changes indicates prothromogenic changes in the blood in patients with NAFLD and an increased tendency to thrombus formation. (9). Given the complex interactions between obesity, NAFLD, and hypertension, the effect of the complex of these diseases on the state of hemostasis requires further research. (9). Therefore, we performed assessment of the influence of obesity on platelet aggregation and blood coagulation in NAFLD and NAFLD, combined with HT. The purpose of this study is to evaluate platelet aggregation and blood coagulation in cases of HT combined with NAFLD under the influence of obesity.

MATERIALS AND METHODS

Participants

The research was conducted at the Bogomolets National Medical University at Department Propaedeutics of Internal Medicine No 1. We examined 152 patients and 15 individuals for control group.

We divided patients by groups:
I group - HT without liver injury – 46 individuals, median and interquartile range of the subjects were 58.00 (51.00; 63.00) years;
II group - NAFLD without HT – 54 individuals, median and interquartile range of the subjects were 54.00 (43.00; 58.00) years;
III group - HT combined with NAFLD (HT+NAFLD) – 52 individuals, median and interquartile range of the subjects were 57.50 (48.00; 64.50) years.

The control group included 15 practically healthy individuals comparable in age and sex, the median and interquartile range of the subjects were 49.00 (42.00; 55.00) years, who underwent a preventive examination.

Laboratory assessment

All patients underwent general clinical examination. A laboratory test included the evaluation of a total platelet count, mean platelet volume (MPV), platocrit (PCT), platelet distribution width (PDW), using an automatic hematology analyzer Mindray BC 2800 (Mindray, China). We evaluated spontaneous and induced platelet aggregation. Platelet aggregation activity was studied using a 230-LA aggregation laser analyzer (Biola Research and Production Company, Russia). Spontaneous and induced platelet aggregation was studied using inducers: ADP – adenosine-5-diphosphate, collagen, AA – arachidonic acid, adrenaline (NPO-Renam, Russia). We also studied blood coagulation activity in the examined patients.

Statistical analysis

Statistical package Portable Statistica 10 (StatSoft, Inc., USA) was used for statistical analysis. Medians and interquartile scale (25th-75th percentile) groups were used. Values of statistical hypotheses with the level of 0.05 ($p<0.05$) were considered statistically significant. We performed statistical analysis of platelet-plasma hemostasis with non-parametric statistical methods: U-Mann-Whitney test, Kruskal-Wallis H-test.

Ethical clearance

The Committee on Bioethics in Bogomolets National Medical University (Ministry of Health of Ukraine) approved the study (expert opinion No 97 dated 23.06.2016)

RESULTS

Among all examined individuals, there were 89 (53%) obese patients (52 (31%) were women and 37 (22%) were men); 78 (47%) patients were not obese (35 (21%) were women and 43 (26%) were men).

To determine the effect of obesity on platelet-plasma hemostasis, the examined groups were divided into two cohorts:
- subgroup with obesity;
- subgroup without obesity.

Thus, among patients with HT 19 (41%) were not obese, and in 27 (59%) patients the BMI was ≥30 kg/m2. There were 30 (56%) obese patients in the NAFLD group and 24 (44%) obese patients. A similar trend was observed in the HT cohort with NAFLD - 30 (58%) patients were obese, in 22 (42%) BMI was <30 kg/m2. Thus, the prevalence of obese patients was observed in all groups of subjects.

Studying the influence of overweight on platelet count determined that in the general cohort the presence of obesity increased the number of platelets in the blood. Obese patient had 12.8% ($p<0.001$) more platelets than non-obese patients, and accordingly PTC were also

9.3% higher in overweight patients (p<0.001).

Significant differences were observed in platelet count and mean platelet volume (MPV) levels, depending on the presence of obesity among patients with isolated course of HT. Platelet count were higher by 18% (p<0.05) among patients with HT and obesity, compared with hypertensive individuals with normal body weight, which reflects the trend in common population. Higher MPV by 6.4% (p<0.05) was also detected in obese hypertensive subjects vs non-obese patients.

In the group of NAFLD patients without HT, obesity affected both - the platelet count and MPV. Platelet count was higher in case of obesity by 16% (p<0.05), MPV was more by 2% in NAFLD subjects with obesity (p<0.05).

Next step was assessment the influence of overweight on platelet aggregation (Fig. 1).

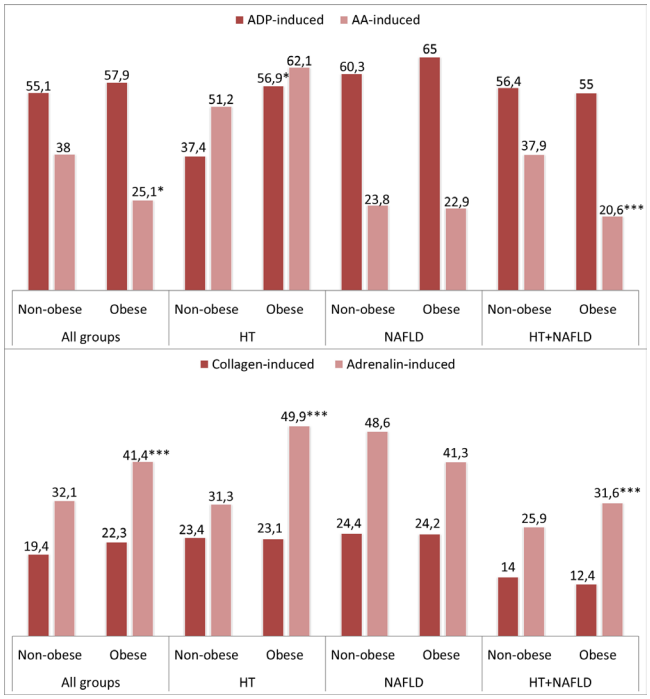

Figure 1: The state of functional activity of platelets in different groups of patients depending on the presence of obesity. Note. The significance level relative to indicators of patients without obesity: * - p<0.05; ** - p<0.01; *** - p<0.001.

Significant differences of platelet functional activity among all patients depending on the presence of obesity were found only in case of stimulation of platelet aggregation with arachidonic acid (AA) and with adrenaline. Thus, the level of AA-induced platelet aggregation was 44% higher (p<0.05) in case of obesity, while adrenaline-induced aggregation was 32% higher (p<0.001) in case of BMI≥30kg/m^2. The degrees of spontaneous platelet aggregation, adenosine diphosphate (ADP)-induced and collagen-induced aggregation did not change under the influence of obesity in the general cohort.

Assessment of the state of platelet aggregation in HT-group showed that the presence of obesity affects the functional activity of platelets. The degree of spontaneous platelet aggregation, AA-induced and adrenaline-induced aggregation remained higher in both subgroups of hypertensive patients, both with obesity and without it. At the same time, the levels of spontaneous and adrenaline-induced platelet aggregation had significant differences due to obesity. The degree of spontaneous aggregation exceeded by 49% (p<0.01) in the obese hypertensive subgroup vs non-obese hypertensive patients. Also, we found out 37.3% (p<0.001) higher level of adrenaline-induced platelet aggregation in obesity with HT. ADP-induced aggregation was higher by 44.3% (p<0.05) in patients with HT and BMI ≥30 kg/m2 compared to non-obese hypertensive individuals.

Analysis of spontaneous and induced aggregation in patients with NAFLD without concomitant HT did not detect contrast between subgroup with and without obesity.

The presence of obesity in the group of patients with combined course of HT and NAFLD did not affect spontaneous, ADP-induced and collagen-induced platelet aggregation. But at the same time, AA-induced aggregation reduced by 45.7% (p<0.001) in obesity against normal body weight in patients with HT+NAFLD. Besides AA-induced aggregation, obesity in these group (HT+NAFLD) affected adrenaline-stimulated aggregation, which enhanced in individuals with overweight (by 18%; p<0.001) against the patients without obesity.

The next step in our study was determination of obesity effect on coagulation hemostasis in groups with HT, NAFLD and their comorbid course (Fig. 2).

After analyzing the coagulation parameters, we found out tendency to accelerate coagulation in obese patients with HT vs non-obese hypertensive individuals. There was a reduction in thrombin time (TT) by 8.1% (p<0.05), raising of fibrinogen by 25.8% (p<0.001) and increasing of soluble fibrin-monomer complexes (SFMC) by 4 times (p<0.001) in obesity. However, prothrombin time (PTT) was significantly lower in patients with HT without obesity - by 8.8% (p<0.01). This proves the complexity and multistage effect of obesity in patients with HT at different levels of the coagulation cascade. There were no effects of obesity on the activation of the internal coagulation pathway by determining the level of activated partial thromboplastin time (APTT).

Interesting was the fact that in patients with NAFLD in both II and III groups, obesity did not lead to significant differences in coagulation parameters, despite the significant differences from the control group in groups without obesity and with a BMI ≥30 kg/m2. At the same time, comparative analysis of the entire cohort of obese

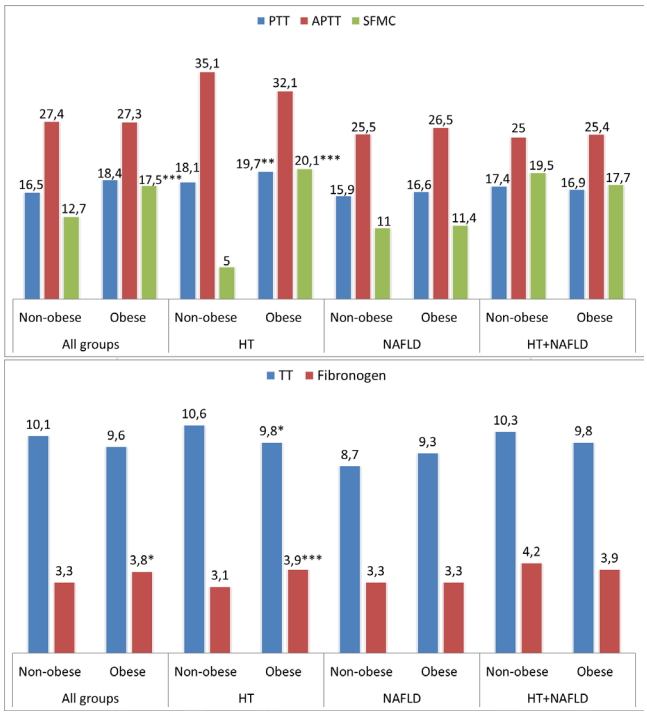

Figure 2: Indicators of coagulation hemostasis in different groups of patients depending on the presence of obesity. Notes. The significance level relative to indicators of patients without obesity: * - p<0.05; ** - p<0.01; *** - p<0.001; APTT - activated partial thromboplastin time; TT- thrombin time; PTT - prothrombin time; SFMC - soluble fibrin-monomer complexes.

subjects showed a difference in fibrinogen and SFMC levels, which were more in subgroups with obesity by 15.2% (p<0.05) and 37.8% (p<0.001) respectively. Thus, the direct effect of obesity was most significant at the final stages of thrombosis.

According to the design of this study, the time of clot formation in platelet-free plasma was analyzed with the highly specific snake venoms. A well-defined point of application of each of the reagents characterizes the individual factors of blood clotting and the different stages of the blood clotting process (Fig. 3)

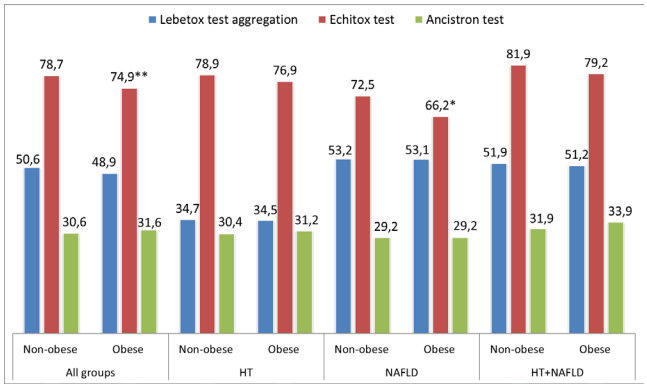

Figure 3: Snake venom test results in different patient groups depending on availability obesity. Note. The significance level relative to indicators of patients without obesity: * - p<0.05; ** - p<0.01.

Evaluation of the thrombosis rate with tests of highly specific snake venoms showed a difference between obese and non-obese patients only by the value of the echitox test (Echus multiscvamosus carinatus). Interestingly, a reduction in clot formation time of 9.5% (p<0.05) was found in obese patients with NAFLD, although evaluation by conventional coagulation parameters (PTT, INR, TT, APTT) did not reveal differences due to obesity in NAFLD group. This deviation was masked in the overall cohort of NAFLD, only the distribution by the presence of obesity demonstrated that patients with NAFLD and BMI ≥30 kg/m2 had a decrease of clot formation time, while NAFLD-patients without obesity had the same values as healthy individuals. The echitox test was also shortened by 5% (p<0.01) in general obese population, which correlates with the results of TT assessment and also indicates an escalation of fibrin making speed in obese subjects.

DISCUSSION

Obesity was accompanied by rising of platelet count in peripheral blood of the general population of patients and an increase in the MPV in patients with HT. Such changes are confirmed by the literature, according to which higher platelet number in visceral obesity is present, also increasing of platelet aggregation presents in obese patients (11). Platelet count is considered marker of inflammation and risk factor of cardiovascular disease (9). However, in non-obese NAFLD patients, the MPV was higher than in obese individuals with NAFLD, that indicates a greater effect of hepatic steatosis on platelet volume. The functional activity of platelets most significantly increases to obesity in patients with HT: increasing the degree of spontaneous aggregation, ADP-induced and adrenaline-induced aggregation. Comorbid course HT and NAFLD also increased the degree of adrenaline-induced platelet aggregation in case of obesity, which can be explained by sympathetic-adrenal system activation in pathophysiologic mechanisms of HT and obesity (4). In the general cohort of the examined patients, the direct effect of obesity is most significant at the final stage of thrombosis formation due to the increasing of fibrinogen and the level of SFMC. In patients with HT hemostatic changes in the blood were also exacerbated by accelerating the transformation of fibrinogen to fibrin. Analysis of the coagulation link of hemostasis with highly specific snake venoms revealed acceleration of fibrin formation in obese patients in the NAFLD group. Chronic mild inflammation caused by obesity may be the partially cause of the increased incidence of thromboembolic complications. Proven active participation in the procoagulant process of increased thrombin production due to excessive release of tissue factor from fat cells, thrombogenic modulation of platelet aggregation caused by leptin and adiponectin, and decreased fibrinolysis due to excessive release of PAI-1 (12). In addition, it has been suggested that enhanced biosynthesis of coagulation factors, including

fibrinogen, may be caused by changes in hepatic metabolism in obesity (13). The balance between clot formation and dissolution determines the maximum coagulation potential, respectively, we have shown that patients has more active coagulation. Coagulation increased due to an increase in both main ways that affect the strength of the clot: fibrin and platelets. It has been shown that obese patients had significantly higher levels of functioning fibrinogen, as well as significant relative thrombocytosis compared with patients with normal body weight, which is consistent with the literature (12,13).

CONCLUSION

Obesity increases blood thrombogenic activity in both HT and NAFLD combined with HT, affecting different stages of hemostasis. Chronic mild inflammation caused by obesity may be partly the cause of thrombophilic changes in the blood. However, platelet aggregation and coagulation in subjects with isolated NAFLD did not contrast in obese individuals and normal weight patients, indicating parallel pathogenic pathway of prothrombotic changes for obesity and NAFLD.

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