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Estimation of platelet activation and factor X activity in plasma of hypertensive patients

Ocena stopnia aktywacji płytek krwi oraz aktywności czynnika X w osoczu chorych na nadciśnienie tętnicze

In the recent decades hypertension became one of the biggest health, social and economic problems. Due to its wide–spread and interdisciplinary character, hypertension arouses special interests among researchers and doctors of various specialties. It is one of the most relevant, controllable risk factors of atherosclerosis and the related cardio–vascular diseases, particularly thrombotic–embolic complications. Despite numerous experimental and clinical studies, the pathomechanism of those complications remains partly unexplained. Although the role of endothelium and its antithrombotic, antiatherosclerotic, antimitotic and vasodilating properties is unquestionable, the mechanisms of interactions between endothelium and blood cells of hypertensive patients and the resulting consequences are less known. The role of platelets in transcellular interactions with endothelium and their involvement in the processes regulating blood hemostasis may be of major importance to the unfavourable thrombotic changes in plasma resulting in various organ complications. Being the biggest endocrine organ, endothelium secretes many substances which regulate: a) tone and hyperplasia (growth) of smooth muscular coat; b) aggregation and adhesion of platelets; c) blood clotting and fibrinolysis; d) adhesion of monocytes and their migration to vascular walls. The dysfunction of endothelium results in the increased vascular resistance and blood clotting as well as faster development of atherosclerotic lesions. In the course of hypertension, endothelial cells are mechanically injured and the extremely thrombogenic subendothelial matrix is exposed. Numerous substances are released which activate platelets and initiate the uncontrollable process of coagulation (8, 16). The thrombocyte activation is connected with the changes of their shape, the formation of pseudopodia and their contact with the site of endothelium injury through the receptors specific for the von Willebrand factor, collagen, fibrinogen, thrombin, serotonin, thromboxane, adrenaline and the platelet–activating factor (13, 17). The process of platelet activation results in the connection of α and dense granules with the cell membrane and the release of several substances: ADP, serotonine and tromboxane A2 which contribute to the recruitment and activation of further platelets (15).

The platelets take part in the secondary hemostatis, i.e. strengthening of platelet plugs by fibrin deposit thanks to the ability of their membrane phospholipids (platelet factor 3) to accelerate clotting activation and thrombin formation.

The aim of the present paper was to evaluate the platelet activation and factor X activity in plasma of hypertensive patients.

MATERIAL AND METHODS

The studied group consisted of 19 patients (5 men and 14 women) with isolated hypertension, without other diseases, aged 36–76, average 61 ± 4.3. The patients qualified for the studies did not smoke or drink, did not take any medicines for at least last 2 weeks, their systolic pressure was ≥ 140 mmHg and/or their diastolic pressure was > 90 mmHg. The control group included 17 healthy volunteers aged 22–68, average 44 ± 3.1.

The examinations were performed between 7 and 9 a.m., in fasting state, in a sound–proof room. The arterial blood pressure was measured in a sitting position after a 15 minutes' rest by means of an indirect method using mercury sphygmomanometer. The venous blood was collected in a recumbent position inserting the needle into a femoral vein. 9 volumes of blood were collected into the syringe filled with 1 volume of anticoagulant (EDTA–50 mg x 0.1 ml + sodium citrate – 40 mg x 0.1 ml, pH = 7.4). The blood samples were centrifuged for 20 minutes at 2000 g. The upper 2/3 of plasma layer was carefully drawn off with a pipette and poured into new polypropylene test tubes. Before the assays, plasma (100 µl) was mixed with 3 ml of 0.05 M Tris–HCl buffer, pH = 7.3.

The platelet factor 3 activity determinations: 0.9 ml of 0.15 M Tris–HCl, pH = 7.4, 0.1 ml of factor IX a + X concentrate solution (Kabi, Vitrum, Molndal, Sweden), 0.1 ml of plasma and 0.1 ml of Russel viper venon solution (Kabi, Vitrum, Molndal, Sweden) were added to the spectrophotometric cuvette (Specord UV–VIS, Karl Zeiss Jena, Germany) kept at room temperature. The mixture was incubated for 1 minute at 37°C and the reaction induced by adding 0.4 ml of the mixture containing 3 parts of 1 mM solution of chromogenic peptide substrate S–2238 (Kabi, Vitrum, Molndal, Sweden) (3) and 5 parts of 0.05 mM $CaCl_2$. The reaction course was followed for 8 min. Observing the curve of extinction value changes at 405 nm (E405).

The factor X activity determinations: 10 µl of plasma and 20 µl of 0.05 M Tris–HCl, pH = 7.8 containing 20 mg/ml of polybren (hexadimetrin bromide). When the solution reached 37°C (3–4 min.) 200 µl of chromogenic tripeptide substrate, S–2337 (1.5 mM in H_2O) (4) were added. Then the mixtures (1:1 vol.) of 0.07 g/l of Russel viper venon (Sigma, St. Louis, USA) and 0.14 $CaCl_2$ were poured at the intervals not longer than 30 seconds. The reaction course was followed on the basis of the amount of p–nitroaniline formed observing the increase of extinction values at 405 nm (E_{405}) for 5 min. The factor Xa activity was expressed in µl kat/l, where 1 katal is defined as enzyme activity releasing 1 mol of p–nitroaniline in 1 second.

RESULTS

In the hypertensive group the average values of systolic and diastolic pressure were 179.05 ± ± 25.69 mm Hg and 104.76 ± 14.36 mm Hg, respectively. Those values in the control group were found to be 124.71 ± 7.80 mm Hg and 78.23 ± 4.11 mm Hg. The comparison of the results in both group is presented in Figure 1.

Table 1 presents the results of platelet factor 3 and factor X activities in control and hypertensive groups.

Figure 2 shows the plasma factor X levels in controls (C) and hypertensive patients (H). The statistically significant increase is observed in hypertensive group compared to controls ($p < 0.01$).

Figure 3 presents the curve of plasma factor 3 levels in controls and hypertensive patients. And similarly, the significantly increased level was observed in hypertensive group compared to controls ($p < 0.01$).

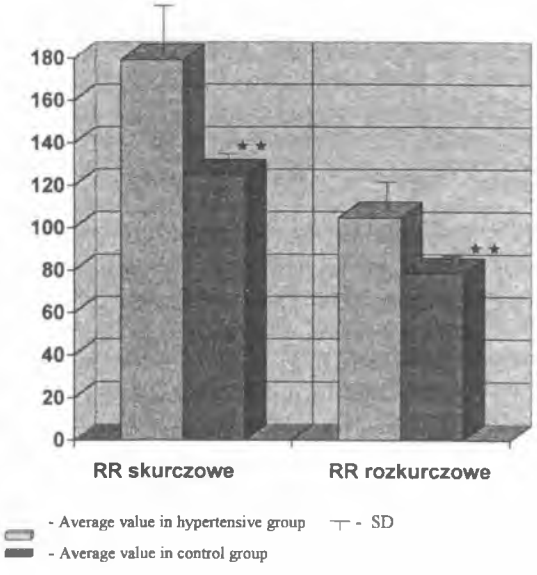

Fig. 1. Systolic and diastolic blood pressure (RR) (mmHg) in hypertensive patients and controls; ** $p < 0.01$

Table 1. Platelet factor 3 and factor X activities in plasma of controls and hypertensive group. Average values ± S.D; ** $p < 0.01$

	Control group n = 17	Studied group n = 19
Factor X (μkat/l)	23.94 ± 1.52	38.95 ± 2.20 **
Platelet factor 3 (ΔE_{405})	0.25 ± 0.01	0.45 ± 0.02 **

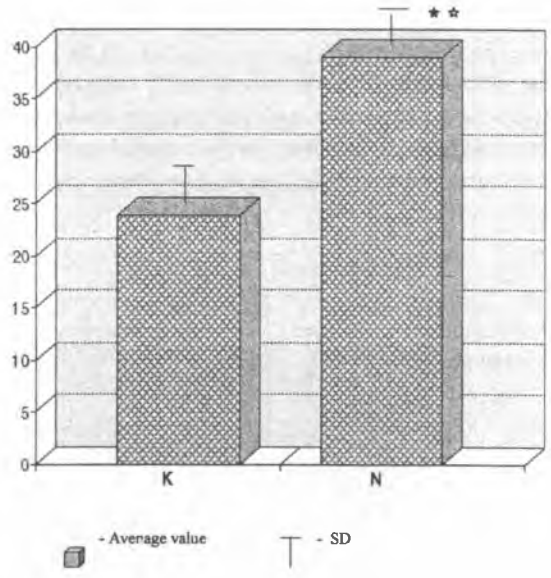

Fig. 2. Plasma factor X levels (μkat/l) in control (K) and hypertensive (N) groups; ** $p < 0.01$

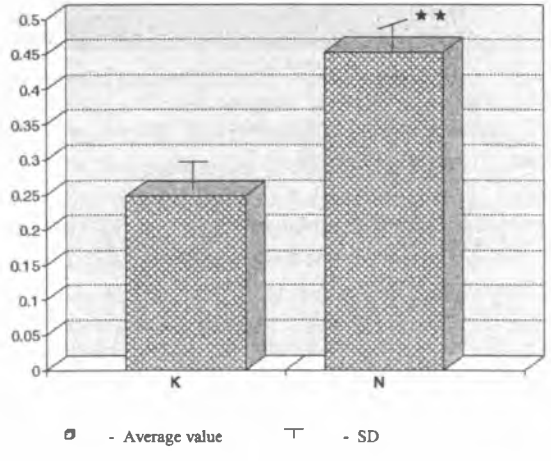

Fig. 3. Plasma factor 3 concentration (E405) in control (C) and hypertensive (H) groups; ** $p < 0.01$

DISCUSSION

Platelets play a key role in the cascade of processes initiated by the endothelium damaged in the course of hypertension. Due to their contact with subendothelial structures, platelets are activated. The simultaneous exposure of the tissue factor from subendothelial structures induces the clotting

factor activation converting further proenzymes into proteases. The most potent coagulation risk factors, platelet factor 3 (PF 3), are the negatively charged phospholipids of the platelet membrane providing the catalytic surface which, in the so–called intraderivative system, accelerates the clotting activity by thousands of times. Those phospolipids constitute about 60–70% of the lipid layer of platelet membrane. Five main classes of phospholipids were identified: phosphatidylethanolamine (PE) – 26–27% of all platelet phospholipids, phosphatidylcholine (PC – 38–39%), phosphatidylserine (PS – about 10%), phosphatidylinositol (PI – 4–5%) and sphingomyelin (17–19%) (1, 7, 11). Those phospholipids are asymmetrically distributed on the outer and inner surface of the cell membrane. When platelets are inactive, the fraction exposed to the outside is composed mainly of Sph and PC (10) while the aminophospholipid exposure (PS and PE) is highly limited. The platelet activation is connected with the PS and PE shifting to the outer membrane surface. The exposure of those classes of phospholipids is identified with the platelet factor 3 availability. The factor is not released to extracellular space but gives the platelet surface its specific properties. The negatively charged phospholipid surface catalizes 2 intermediate reactions in the intraderivative cascade of clotting, i.e. activation of factor X to Xa by means of factors IXa, VIIIa and Ca2+ (forming tenase) and activation of prothrombin to thrombin by means of Xa, Va and Ca2+ (prothrombinase) (5).

The results of the platelet factor 3 activity determinations performed in control and hypertensive groups showed the statistically significant increase of this factor in hypertensive patients ($p < 0.01$). In the prethrombotic state, the constant, slight aggregation and degranulation of platelets is suspected. Similar phenomena result from vascular wall injuries occurring in hypertension (6) or in atherosclerotic lesions. Among few papers evaluating the degree of platelet activation in hypertensive patients, there is a report of Nityanand et al. (12) in which the changes in morphological structure and the platelet clotting activity risk were studied in 39 patients. The increased platelet activity was observed which correlated with higher values of diastolic pressure. Andrioli et al. (2) who studied the platelet activity in 21 hypertensive individuals also found the increased thrombocyte activity in hypertensive patients compared to normotensive controls. Lupu et al. (9) evaluating the changes in platelet membrane phospholipids observed the significant PF 3 activity increase in diabetic patients, which resulted in the increased clotting activity risk in plasma.

The present paper studied the activity of clotting system in hypertensive patients in comparison with the group of healthy individuals by determining the factor X concentration. The highly significant increase in the factor X level was found in the hypertensive group ($p < 0.01$). According to the definition introduced by Bauer and Rosenberg (4), the biochemical marker of prethrombotic state is the increased factor X activity with normal or slightly increased factor II activity. Thus, our own studies concerning the factor X activity seem to give further evidence proving that hypertension is related to the state of thrombotic readiness.

CONCLUSIONS

1. The increased platelet activation is observed in hypertensive patients compared to healthy individuals. This is visible in the statistically significant increase in the platelet factor 3 activity which may intensify the risk of higher aggregative and coagulative activity of plasma.

2. In hypertensive patients, the significant increase in plasma factor X activity is found which is likely to be related to the state of thrombotic readiness.

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STRESZCZENIE

Celem pracy była ocena stopnia aktywacji płytek krwi oraz aktywności czynnika X w osoczu chorych na nadciśnienie tętnicze. Grupę badaną stanowiło 21 chorych z izolowanym nadcisnieniem tętniczym, a grupę kontrolną 17 zdrowych ochotników. Aktywność czynnika płytkowego 3 oraz czynnika X w osoczu badanych określano przy użyciu syntetycznych trójpeptydowych substratów chromogennych. U chorych z nadciśnieniem tętniczym stwierdzono istotny statystycznie wzrost stężenia czynnika płytkowego 3 oraz czynnika X, co może nasilać proagregacyjną i prokoagulacyjną czynność osocza.