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Department of Internal Diseases and Endocrynology
Cardinal Stefan Wyszyński Specialist Hospital in Lublin
Department of Clinical Dietetics Faculty of Nursing and Health Sciences
Medical University of Lublin

MAŁGORZATA TOMCZYKOWSKA, JANUSZ BIELAK, ARTUR BODYS

Evaluation of platelet activation, plasma antithrombin III and α_2-antiplasmin activities in hypertensive patients

Due to its common and interdisciplinary character, arterial hypertension belongs to the diseases arousing much interest among scientists and physicians of various specialties. The results of numerous statistical studies show that the problem concerns about 20–40% of the population. Arterial hypertension is one of the most important, permanent, controllable and modifiable risk factors of atherosclerosis and associated cardiovascular diseases, particularly so common thrombotic-embolic complications. Despite the comprehensive studies, the patho-mechanism of such complications has not been fully explained yet. The mechanisms of interactions between the endothelium and blood cells of the hypertensive patients as well as their consequences are poorly known. The role of platelets in transcellular interaction with the endothelium and their involvement in haemostasis-regulating processes may play a key role in plasma thrombotic changes that are likely to result in numerous organ complications.

The platelets are vital elements of the cascade of processes initiated by the injured endothelium in arterial hypertension. The exposed connective tissue of blood vessels is an extremely thrombogenic environment. On contact with subendothelial structures, the platelets undergo activation, changing their disc shape into the spherical one. The simultaneous exposure of the tissue factor from the subendothelial structures initiates the activation of the clotting factors, converting the successive pro-enzymes into active proteases. The most potent procoagulative factors of platelets, called platelet factors 3 (PFs 3) are negative phospholipids of their cell membrane forming the catalytic surface which increases the rate of clotting activation in the endogenous system by hundreds of times. The negatively charged phospholipid membrane surface catalyses two transient reactions in the endogenous cascade of coagulation, i.e. activation of X to Xa by active factors IXa, VIIIa and Ca^{2+}(tenasis) and activation of prothrombin to thrombin by active factors Xa, Va and Ca^{2+} (prothrombinase). Phospholipids increase the X and II activation by binding the proteins with lipid surface, which highly intensifies the local concentration of the enzyme and substrate. PF3 becomes available only during the platelet activation, and therefore the determinations of intensity of its occurrence may be a good indicator to study this process.

In the physiological conditions, a relevant element of the haemostatic system limiting the clotting activation is the natural anticoagulative system whose actions trigger the generation of thrombin. This system consists of 3 groups of coagulation inhibitors. The first group includes: thrombomodulin (TM) - the thrombin receptor on the cell surface and 2 proteins – C (PC) and S(PS). Thrombin bound by TM completely changes its substrate specificity, looses its ability to

convert fibrinogen and activate the factors VII, V, XIII and platelets. The ability of thrombin to activate PC increases by a thousand times after its binding with TM. The active protein C is an anticoagulative serine proteasis which digests and inactivates the factors Va and VIIIa. This reaction takes place very fast on the phospholipid surface of platelets in the presence of a cofactor – protein S. PS shows high affinity to phospholipids so it may successfully compete with other clotting factors. The active PS stimulates fibrinolysis by inactivating the plasminogen activation inhibitor (PAI-1). Therefore, thrombin, which in its free state acts prothrombotically, once bound with TM becomes anticoagulative and profibrinolytic. The second group of anticoagulants includes endogenous antithrombin III (AT III) and the second heparin cofactor (HC II). AT III binds and inactivates all the clotting proteases, except VIIa, particularly Xa and thrombin. The third group of endogenous coagulation inhibitors consists of the exogenous coagulation system inhibitors.

All the elements of the fibrinolytic system may be divided into 2 groups and their concentration and activity are maintained within fixed, fairly constant ranges. The first group contains enzymatic proteins, which affect the balance leading to haemorrhagic tendencies. These proteins include: plasminogen, the tissue plasminogen activator (t-PA), the urokinase-like activator (u-PA), prekallikrein, the factor XIIa. The second group is composed of antifibrinolytic inhibitors: the plasminogen activator inhibitor – type 1 and 2,- α2-antiplasmin, α2-macroglobulin, the esterase inhibitor C1 and the protein rich in histidine.

The purpose of the study was to evaluate the degree of platelet activation and plasma antithrombin III and α2-antiplasmin activities in hypertensive patients.

MATERIAL AND METHODS

The examinations were performed in the group of 21 patients with arterial hypertension (AH) with systolic pressure ≥140 mm Hg and/ or diastolic pressure ≥90 mm Hg, aged 36–76 (average 63±4.4) and affected by other diseases. The examined group included the patients on a normal diet, non-smokers and non-drinkers who did not take any medicines for at least 2 weeks prior to the examination. The control group consisted of 19 healthy volunteers, aged 22–68, average 56±3.5 years.

In order to eliminate any incidental factors and to standardize the material collection, all the patients were subjected to the same procedure. The arterial pressure was taken in the sitting position after a 15-minute rest, indirectly, using a mercury sphygmomanometer and the Korotkow's method. The samples of plasma rich in platelets were used to determine the activity of PF 3, antithrombin III and α2-antiplasmin. The samples were collected in the recumbent position from the femoral vein. The values of platelet and plasma coagulation factors were determined using the synthetic chromogenic substrates and the spectrophotometric method to measure the amount of p-nitroaniline released by the examined factors. The quantity of the generated p-nitroaniline was determined measuring the rate of an extinction increase at 405nm. The statistical analysis was performed using Statistica for Windows; $p<0.05$ was assumed to be statistically significant.

RESULTS

The table presents the activity values of the selected plasma coagulation factors and platelets in the control and hypertensive groups.

Table 1. Activities of the selected plasma coagulation factors and platelets in the control (K) and hypertensive (N) group. Average values ±S D, *p<0.05, ** p<0.01 N vs. K

	Control group n=17	Hypertensive group n=21
Antithrombin III (U/l)	219.29±5.39	179.00±3.22*
α_2-antiplasmin (ΔE_{405})	21.65±1.27	14.19±0.98*
Platelet factor 3(ΔE_{405})	0.25±0.01	0.45±0.02**

Figure 1 shows the plasma antithrombin III level in the control and hypertensive groups. This level was statistically significantly lower in the hypertensive patients compared to the controls (p<0.05). Antithrombin III (AT III) is the biggest, natural endogenous inhibitor of serine proteases of thrombin and factor Xa and, to a smaller degree, of factors XIIa, XIa and IXa. The inactivation of the above-mentioned enzymes in plasma is very fast. In the prothrombotic conditions found in arterial hypertension, large amounts of AT III are bound with these factors and the inactivated complexes formed. The level of free, available AT III decreases. AT III is excessively used in the inhibition process of the excessively used in the in-

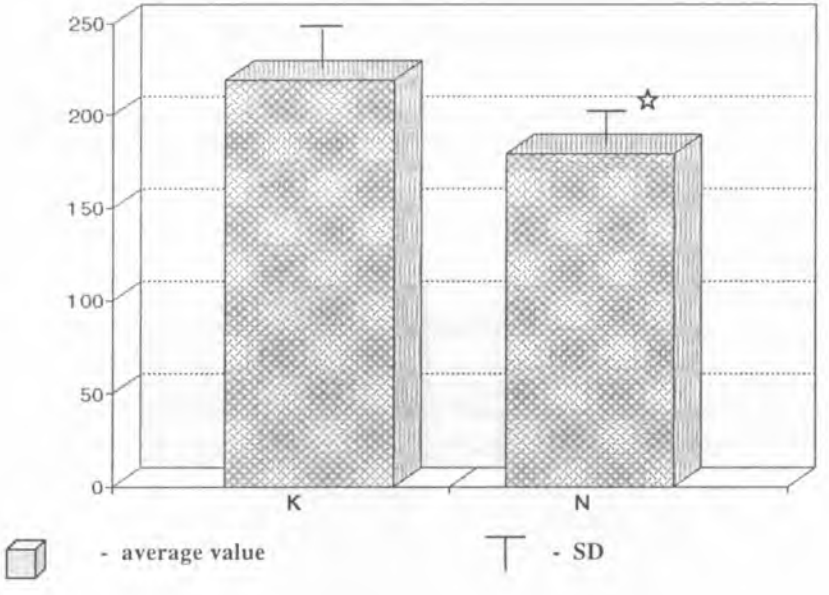

Fig.1. Plasma antithrombin III concentration (U/l) in control (K) and hypertensive (N) groups; *p <0.05

hibition process of the excessively increased uncontrollable procoagulative activity of plasma.

Figure 2 presents the plasma α_2-antiplasmin levels in the controls and hypertensive patients. Compared to the controls, the hypertensive patients showed statistically significantly decreased levels of this factor (p<0.05). This can be explained in two ways. Firstly, increased

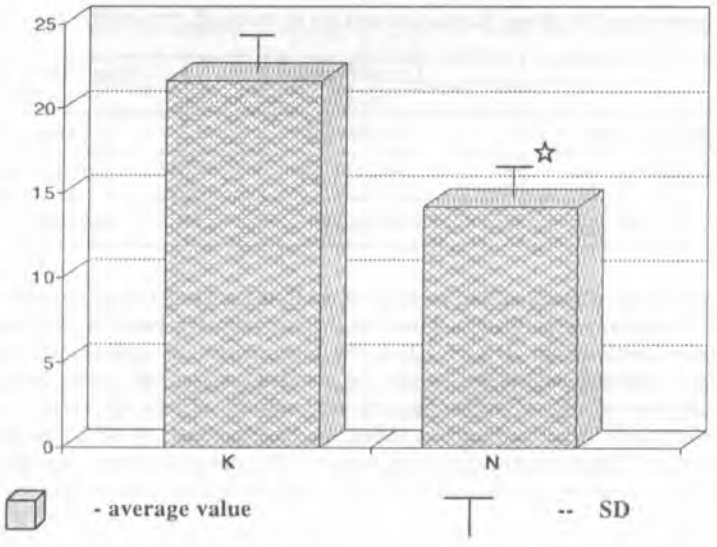

Fig. 2. Plasma α_2 antiplasmin concentration (ΔE_{405}) in the control (K) and hypertensive (N) groups; *p<0.05

procoagulative activity of plasma results in an adequate activation of the fibrinolytic activity, and the excessive plasmin generated secondarily after the lysis of clots is inactivated by α_2-AP

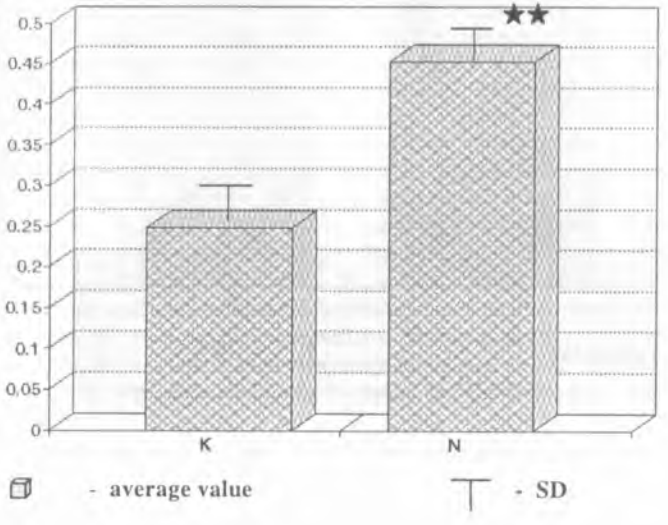

Fig. 3. Plasma platelet factor 3 concentration (ΔE_{405}) in the control (K) and hypertensive (N) patients; **p<0.01

(forming the plasmin-antiplasmin complexes). In this process the reserves of α_2-antiplasmin are completely used and its plasma concentration decreases. Another explanation may be the fact that PAI-1 activity in the hypertensive patients, which inhibits the plasmin generation results in its low concentration and secondarily in low levels of its natural inhibitor.

Figure 3 presents the plasma levels of platelet factor 3 in the controls and hypertensive patients. Compared to the control group, the hypertensive patients show statistically significantly increased levels of this factor ($p<0.05$).

CONCLUSIONS

1. Compared to the healthy individuals, the hypertensive patients show increased platelet activity confirmed by a statistically significant increase in the platelet factor 3 activity, which is likely to intensify the plasma proaggregation and procoagulation activities.

2. In the hypertensive patients, a significant decrease in antithrombin III (the endogenous inhibitor of the coagulation system) concentration is observed.

3. In arterial hypertension, a significant decrease in α_2-antiplasmin activity is found, which may reflect decreased fibrinolytic activity of plasma in the hypertensive patients.

The above findings reveal that the patients with arterial hypertension increased proaggregation activity of platelets and elevated antifibrinolytic activity of plasma, which is likely to underlie the thrombotic-embolic complications developing in this disease. The studies performed suggest that the drugs protecting the endothelium and inhibiting the activity of thrombocytes as well as the remaining elements of the coagulation system cascade should be more widely used.

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SUMMARY

The aim of the study was to evaluate the degree of platelet activation and plasma antithrombin III and α2-antiplasmin activities in hypertensive patients. The studied group consisted of 21 patients with hypertension. The control group included 19 healthy volunteers. The values of platelet and plasma coagulation factors were determined using the synthetic chromogenic substrates and the spectrophotometric method. Compared to the healthy individuals, the hypertensive patients show increased platelet activity confirmed by a statistically significant increase in the platelet factor 3 activity, which is likely to intensify the plasma proaggregation and procoagulation activities. In the hypertensive patients, a significant decrease in antithrombin III (the endogenous inhibitor of the coagulation system) concentration is observed. In arterial hypertension, a significant decrease in α_2-antiplasmin activity is found, which may reflect decreased fibrinolytic activity of plasma in the hypertensive patients.

Ocena stopnia aktywacji płytek krwi oraz aktywności antytrombiny III i α_2 - antyplazminy w osoczu chorych na nadciśnienie tętnicze

Celem pracy była ocena stopnia aktywacji płytek krwi oraz aktywności antytrombiny III i α_2 antyplazminy w osoczu chorych na nadciśnienie tętnicze. Grupę badaną stanowiło 21 chorych z izolowanym nadciśnieniem tętniczym, a grupę kontrolną 19 zdrowych ochotników. Aktywność czynnika płytkowego 3 oraz antytrombiny III i α_2-antyplazminy w osoczu badanych określano przy użyciu syntetycznych trójpeptydowych substratów chromogennych. U chorych z nadciśnieniem tętniczym stwierdzono istotny statystycznie wzrost stężenia czynnika płytkowego 3 oraz obniżenie aktywności antytrombiny III i α_2-antyplazminy, co może nasilać proagregacyjną oraz osłabiać fibrynolityczną czynność osocza.