HEMORHEOLOGICAL AND OXYGEN FREE RADICAL INVESTIGATIONS IN CARDIOVASCULAR DISEASES

Ph.D. thesis

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LIST OF ABBREVIATIONS

ACE	angiotensine converting enzyme
ADP	adenosine diphosphate
AI	aggregation index
ASA	acetylsalicylic acid
CAD	coronary artery disease
CI	cardiac index
CRI	circulatory index
GPX	glutathione peroxidase
GSH	reduced glutathione
GSSG	oxidized glutathione
IHD	ischemic heart disease
HCT	hematocrit
LORCA	laser-assisted optical rotational cell analyzer
PPP	platelet poor plasma
PRP	platelet rich plasma
PTCA	percutaneous transluminal coronary angioplasty
PV	plasma viscosity
RBC	red blood cell
SOD	superoxide dismutase
TBARS	thiobarbituric acid reactive substances
WBC	white blood cell
WBV	whole blood viscosity

1. INTRODUCTION

Cardiovascular diseases are the most frequent cause of morbidity all over the world. Investigations explored the role of vascular wall, cellular interactions, and many of the underlying biochemical processes. Much less attention was paid to the properties of the circulating blood. Hemorheology is the science of blood flow and deals with the deformation and flow properties of blood and its elements under mechanical forces. Blood is a non-Newtonian mixture and its viscosity is shear-dependent, which means that its apparent viscosity increases at low flow rates and decreases with the increase of shear stress. Another phenomenon that makes hemorheology more complex is the biological interactions of the suspended particles. The non-Newtonian behavior of blood is mainly resulted from the presence of red blood cells. Almost every property of this cell modifies the apparent viscosity of whole blood. The strong correlation of the red cell concentration to the viscosity has been demonstrated in numerous studies. Besides hematocrit, size, shape and internal viscosity of red cells can affect viscosity. In healthy state the influence of the other cellular elements, like leukocytes and platelets on whole blood viscosity is negligible, but in diseased states when their number (e.g. in leukemias) or activation (e.g. in ischemia) increases the role of these cells also grows.

Interactions between cellular components of blood such as aggregation, agglutination or adhesion of cells are observed in a variety of normal and pathological conditions. While the agglutination of red blood cells is caused by specific binding of immunoglobulins, the aggregation of the cells (rouleaux formation) is caused by non-specific action of fibrinogen and some other big molecules of blood plasma. This rouleaux formation can also be seen, if red blood cells are resuspended in solutions containing macromolecules like dextran. This type of aggregation of RBCs either in blood or in suspensions is a reversible process; disaggregation can be achieved by shearing of the fluid. There exist two hypotheses explaining the mechanism of red blood cell aggregation process. The bridging hypothesis implies molecular bridges between adjacent cells. The depletion model is coming from colloid chemistry and is based on the depletion of the macromolecules from the interparticular (intercellular) zone. As a consequence, this is accompanied by the decrease of the osmotic pressure in this zone and an increase in the bulk phase of the macromolecules. This leads to the so-called depletion interaction and to the formation of reversible flocks of particles (cells). Red blood cell aggregation is a dynamic process that is also affected by shear stress, membrane properties, and cell deformability. Red blood cell

aggregation is an important component of whole blood viscosity, especially at low shear rates, for instance, in capillaries or post-capillary venules, therefore the characterization of this process can provide useful information.

Ischemic heart disease is mainly caused by coronary artery disease, the stenotic lesion of one or more coronary branches. In the coronary circulation both hemodynamic and hemorheologic factors play an important role. Hemorheological parameters were proved to be primary cardiovascular risk factors in several studies (Framingham Study, Puerto Rico Heart Health Program, Honolulu Heart Program, Northwick Park Study, Monica Project, Edinburgh Artery Study, Caerphilly and Speedwell Collaborative Heart Disease Study, and Physicians' Health Study). Coronary vessel system is a special part of the circulation, since there is a continuous change in blood flow, perfusion pressure, and shear rate due to the cardiac cycle, plus narrowest capillaries of the body can be found in the myocardium (3-5 μm). Therefore the role of rheological alterations may be of higher importance than in other parts of the circulatory system. Previous studies involved small groups of patients, thus further investigations should reveal whether any of the hemorheological factors are in correlation with the stages of CAD, and if so, which of these parameters shows the correlation.

Percutaneous transluminal coronary angioplasty is a widely used method in the treatment of significant coronary stenoses. The high (30-40 %) ratio of reocclusion and restenosis is the main challenge of this procedure. The mechanism of these processes involves platelet and leukocyte activation, thrombus formation, endothelial and smooth muscle cell proliferation, and local and systemic inflammation, but the pathogenesis is still only partially understood. Extreme flow conditions can occur proximal to, through and distal to the coronary stenosis with continuous change in shear stress. At normal circumstances coronary flow is mainly determined by hemodynamic factors, but in critical conditions effects of blood rheology increases. It can also play a role in the deterioration of blood flow in small vessels and may contribute to the slower restoration of coronary circulation even after a successful procedure. The effects of coronary angioplasty on parameters characterizing blood flow properties and on characteristics of erythrocytes have not been clarified.

Oxygen derived free radicals are suggested to have pathophysiological role in a variety of diseases, like atherosclerosis, diabetes mellitus and chronic inflammatory processes. Free radicals are highly reactive chemical species with the potential to damage cellular proteins, nucleic acids,

carbohydrates, and lipids, which can result in cell necrosis. Free radicals are generated during the normal cellular metabolism and cells have various defense systems to protect themselves. These systems involve scavenger enzymes (superoxide dismutase, catalase, glutathione peroxidase) and several molecules, among these thiol-group containing substances, e.g. glutathione. Oxidative stress following ischemia and reperfusion of the myocardium results in the generation of free radicals in endothelial, myocardial and blood cells that have a great impact on cellular characteristics and interactions, hemorheological conditions, and tissue damage. Elevated free radical activity was reported in patients with coronary artery disease without predicting value on the severity of the disease. Oxidative stress was also suggested as a pathophysiological mechanism in the development of myocardium damage in acute myocardial infarction and heart failure. Nevertheless, there has been relatively limited number of studies, which explored this issue during coronary interventions and the results were not unequivocal.

Although the role of cellular activation has also been discussed several times, the hemorheological aspects of this field are yet to be elucidated. Because of larger diameter and lower deformability, white blood cells can have serious influence on microcirculation. The entry of a WBC into a narrow capillary reduces flow behind the cell. Therefore, only plasma can overtake the WBC and a train of red blood cells can be formed behind the cell. It is also conceivable that a slight difference in flow in two branches can cause all the RBCs to enter into the branch with the higher flow. Only those RBCs with the highest deformability have any chance to pass by WBCs. Therefore evidences of leukocyte activation during myocardial ischemia and reperfusion along with the probable alterations in platelet function and red blood cell properties could imply the severe impairment of coronary circulation. This impairment does not necessarily take place only during ischemia, but it can be a causal factor in "no-reflow" phenomenon.

Diabetes mellitus is the most frequent cause of blindness and renal failure and a risk factor of coronary, cerebral and peripheral artery diseases. Both insulin dependent and non-insulin dependent types of diabetes are complicated with micro- and macroangiopathies. Several metabolic disturbances are involved in the development of these complications: glycosilation of basal membrane in the vascular wall resulting in the reduction of elasticity and the increase of permeability, glycosilation of LDL particles that can promote atherogenesis, activation of platelets, reduction in fibrinolytic activity, elevation of von Willebrand factor concentration, smooth muscle cell proliferation due to the mytogenic effect of hyperinsulinaemia. Recently, the attention was attracted to hemorheology. Many changes that can be seen in diabetic retinopathy

(dilated veins, microaneurysms, hemorrhages, vessel proliferation) could also be observed in experiments when blood viscosity was increased or in diseases with high blood viscosity (e.g. sickle cell disease, leukemia, macroglobulinemia). Microvascular damages affect the vessels of the retina, the myocardium, the kidney, the skin and the nervous system more severely. Most of the previous studies found elevated whole blood viscosity in diabetic patients, although results were equivocal if the elevation of this parameter correlated with serum glucose concentration or the complications, e.g. retinopathy and whether metabolic control ameliorated blood viscosity also. Plasma viscosity was mainly measured higher in diabetics, but some studies found no difference. Red cell deformability measurements found decreased filterability with filtration techniques and slower shape recovery with micropipette method. Although the former observed changes were not significant in some investigations when white blood cells, erythrocyte and platelet aggregates, fibrin plugs causing filter pore clogging were removed from the samples.

2. AIMS OF THE INVESTIGATION

1./ Examination of microrheological characteristics of blood is a really challenging field of hemorheology. Our interest includes evaluating red blood cell aggregation measurements in the clinical practice by different methods. The effect of plasma fibrinogen concentration of human blood on red blood cell aggregation was examined using two optical aggregometers. The results obtained by the devices were compared to each other also in order to test, which is more reliable in the clinical practice.

2./ Our purpose was to investigate the hemorheological characteristics of patients with ischemic heart disease. The relation of the severity of coronary artery disease and hemorheological factors was examined in patients who underwent coronary angiography. Central hemodynamic parameters were also measured and the circulatory index (CRI) characterizing the hemodynamic and the hemorheologic state of the circulation in one formula was calculated.

3./ Hemorheological alterations could be of crucial importance during coronary interventions when coronary circulation is affected not only by stenotic lesions, but also balloon inflations and vascular injury. The aim of this study was to investigate whether hemorheological parameters change during and after PTCA. There has been relatively lower number of studies which tried to follow up laboratory parameters several days and months

after a coronary event or a coronary intervention, despite that vessel wall remodeling and restenosis can develop during this longer period. Therefore we determined the hemorheological parameters on several days after PTCA and at the first and sixth month's regular visit also.

4./ Besides hemorheological alterations, oxygen derived free radicals can also play a part in the pathologic processes after PTCA. Effects of oxidative stress and oxygen derived free radical mediated damage were investigated determining lipid peroxidation, activity of antioxidant systems and cellular (neutrophil granulocyte and platelet) activation in patients undergoing coronary angioplasty.

5./ Macro- and microrheological parameters of blood were investigated in patients suffering from diabetes mellitus complicated with retinopathy. Red blood cell aggregation was studied by two methods. Data of patients with non-proliferative and proliferative retinopathy was compared to each other.

3. METHODS

3.1 Hemorheological measurements

Hematocrit, plasma and whole blood viscosity

2×4.5 ml blood samples were collected into lithium-heparin coated Vacutainer tubes for hematocrit, plasma viscosity, whole blood viscosity, and red blood cell aggregation measurements. Hematocrit was measured by centrifuging hematocrit capillaries at 12000 rpm for five minutes in microhematocrit centrifuge (Hemofuge, Heraeus). Plasma was prepared by centrifuging one tube of blood at 1500 g for ten minutes. Plasma and whole blood viscosities were determined in Hevimet 40 capillary viscosimeter (Hemorex, Hungary). 0.5-0.5 ml of plasma or whole blood was injected into the capillary tube of the device. In this viscosimeter the flow of the fluid is detected optoelectronically along the capillary tube and a flow curve is drawn. Shear rate and shear stress are calculated from this curve by a computer program. Viscosity values are determined as a function of these parameters according to Casson's principle. For the presentation of our results, apparent whole blood viscosity values calculated at 90 s^{-1} shear rate are given. Corrected whole blood viscosity was calculated with a mathematical formula according to Mátrai et al. In this formula apparent whole blood viscosity value at 90 s^{-1} shear rate (WBV_{HCT}) is used and correction is made to 40% hematocrit:

$$WBV_{40\%} / PV = (WBV_{HCT} / PV)^{(40\% / HCT)}$$

Hematocrit measurement was performed at room temperature (22±1 °C), viscosity measurements were carried out at 37 °C within three hours after venepuncture.

Plasma fibrinogen

4.5 ml blood sample was drawn into a Vacutainer tube containing sodium citrate (0.129 M, 1:10 dilution) and plasma fibrinogen concentration was determined by using Clauss's method.

Red blood cell aggregation

Various techniques exist in examining red cell aggregation like erythrocyte sedimentation, blood viscosity measurements at low shear stress, optical methods, or micromechanical (cellular) methods. Among these, optical aggregometry is the most widely used technique, which is based upon the alteration in the light intensity transmitted through or scattered back from blood. The Myrenne aggregometer is the previously developed and most frequently used optical aggregometer in hemorheological laboratories. In this device 20-25 µl of anticoagulated whole blood is placed between a transparent rotating flat (2°) perspex cone and a stationary plate and the sample is disaggregated by shearing at 600 s^{-1} for 10 seconds, which followed by the sudden stop or low shearing at 3 s^{-1} of the sample (M and M1 modes). The change of infrared light transmission is converted into an aggregation parameter: AI_M or AI_{M1}, respectively. Measurements are carried out at room temperature (22 ± 1 °C).

In the recently developed Laser-assisted Optical Rotational Cell Analyzer basic principle of detection of RBC aggregation is similar to that in Myrenne, namely, light intensity changes are converted into aggregation index. In this system a diode laser is the light source and the light scattered back from whole blood placed in the 400 µm gap between the walls of cup and bob is sensed by photo diode sensors. Aggregation index is calculated as a ratio of the area above the syllectogram and the total area over a fixed time period. In LORCA temperature can be adjusted accurately to 37°C, which creates more physiological conditions for the aggregation process. 2 ml of whole blood (the same sample was used as in whole blood viscometry) was injected into the cup of LORCA; detection of laser back-scattering from the sheared (disaggregated), then unsheared (aggregating) blood was performed in a computer system. Aggregation index (AI_L) was calculated from the light intensity curve on the basis that there is less light scattered back from aggregating red cells. With LORCA aggregometer it is also possible to determine the minimum disaggregation shear rate ($\gamma_{disc-min}$) by adjusting several shear rates and measuring the accompanying light backscatter, which

represents the minimum shear force that is necessary to separate red blood cells from each other. All aggregation measurements were done within three hours after venepuncture. Aggregation indices were correlated to each other, to plasma fibrinogen concentration, and to plasma viscosity. Minimal disaggregation shear rate was also correlated to plasma fibrinogen concentration and to plasma viscosity.

3.2 Determination of free radical associated parameters

Thiobarbituric acid reactive substances

The oxidative degradation of polyunsaturated fatty acids containing at least three double bonds results in the formation of malondialdehyde. The aldehyde group of malondialdehyde reacts with thiobarbituric acid to form a pink-colored complex. As there are other molecules that can react with TBA, this group of molecules is named thiobarbituric acid reactive substances. Blood samples drawn into EDTA containing tubes were analyzed for TBARS in blood as a marker of lipid peroxidation using spectrophotometry at the maximum absorption wavelength of 532 nm.

Activity of the antioxidant system

Changes in the antioxidant system were investigated by measuring the activity of superoxide dismutase, catalase, glutathione peroxidase, and reduced glutathione. Activity of superoxide dismutase of blood and plasma was measured by utilizing the effect of the enzyme in inhibiting superoxide-induced formation of adrenochrome from adrenaline as described by Misra and Fridovich. Activity of catalase was determined spectrophotometrically at 240 nm upon the consumption of hydrogen peroxide and the results were expressed in Bergmeyer units (1 Bergmeyer unit (BU) = degradation of 1 gram of hydrogen peroxide in a minute). Glutathione peroxidase activity of red blood cells was measured by the Chiu-Stultz method. Concentration of reduced glutathione of blood was measured using Ellman's technique.

Superoxide generating capacity of polymorphonuclear leukocytes

Superoxide production of granulocytes was measured according to the method of Guarnieri et al. The method is based on the capacity of superoxide to react with the iron in cytochrome c at rest and after stimulation with phorbol-myristate-acetate. Measurement was carried out in spectrophotometer at 550 nm wavelength. The results were expressed in nanomoles of superoxide anion of 1.5 million neutrophils a minute.

Platelet aggregation

Spontaneous, epinephrine, ADP and collagen induced aggregation of platelets was analyzed. Blood was taken into tubes containing sodium citrate. Samples were centrifuged at 1000 rpm/min for 10 minutes to produce platelet rich plasma, which was carefully removed for measurement; and then centrifuged further at 2200 rpm/min for 15 minutes to get platelet poor plasma. 0.5 ml PRP was measured against 0.5 ml PPP to determine spontaneous platelet aggregation. 25 µl of ADP, epinephrine or collagen was added to PRP so as to measure induced platelet aggregation. Platelet aggregation was measured in Micron M304 aggregometer.

3.3 Impedance cardiography

Impedance cardiography was performed to determine cardiac index (ICG-M401, ASK, Hungary). This parameter and apparent whole blood viscosity value at 90 s^{-1} was used to calculate circulatory index according to the following formula:

Circulatory index *(l/N/s²)* **= *Cardiac index*** *(ml/s/m²)* **/ *WBV*** *at 90 s^{-1} (mPas = mNs/m²)*

3.4 Study populations

Comparative examination of red blood cell aggregation

Sixty blood samples from subjects including healthy volunteers, ischemic heart disease and diabetic patients (mean age: 51 ± 16 years) were analyzed paralelly in Myrenne MA-1 RBC aggregometer and LORCA aggregometer. Hematocrit, plasma fibrinogen, plasma and whole blood viscosities of the samples were also determined. Aggregation indices, namely AI_M, AI_{M1} and AI_L were correlated to each other, plasma fibrinogen concentration, and plasma viscosity. Correlation coefficients were calculated with SPSS statistical analysis software.

Examination of coronary artery disease

162 ischemic heart disease patients (mean age: 55 ± 10 years) consecutively admitted to our department and 59 healthy persons (Group 0 (G-0), mean age: 35 ± 10 years) were examined. All the patients had angina pectoris in their clinical history. After the non-invasive diagnostic procedures (ECG, echocardiography, stress tests, myocardial perfusion scintigraphy), which proved the IHD, all patients underwent coronary angiography. They were classified into

three groups according to their coronary vessel state based on the coronary angiogram. The first group (G-1) included 31 patients without significant coronary artery disease (less than 70% stenosis) in spite of the positive non-invasive tests. Patients with definite coronary artery spasm or coronary X syndrome (small vessel disease) also belonged to this group. In the second group (G-2) there were 29 patients with significant lesion (stenosis or occlusion) of one vessel. In the third group (G-3) there were 102 patients with severe multiple stenoses or occlusions of more than one coronary arteries (double and triple vessel disease). All of the patients were on combined antianginal-antiischemic drug therapy, including nitrates, beta-blockers, calcium-antagonists, ASA, ACE-inhibitors, lipid-lowering agents. Blood samples were taken from the cubital vein, and routine blood chemistry and hemorheological parameters - hematocrit, plasma fibrinogen level, plasma and whole blood viscosity - were determined. Impedance cardiography was performed at the resting subjects in supine position to determine cardiac index. Circulatory index was calculated according to formula described above.

Percutaneous transluminal coronary angioplasty

Nineteen patients (mean age: 58 ± 9 years) were enrolled in this study. These patients had significant stenosis of one main coronary artery (single vessel disease). After coronary angiography, which revealed the site of the culprit stenosis, percutaneous transluminal coronary angioplasty was performed. Patients were on combined antianginal-antiischemic drug therapy (including nitrates, β-blockers, angiotensine converting enzyme inhibitors, calcium antagonists, lipid-lowering drugs and conventional antiplatelet therapy: acetylsalicylic acid, ticlopidine) and heparin was administered as an anticoagulant during the procedure and for 24 hours after PTCA. Coronary artery branch with the significant stenosis was dilated with balloon catheter, which was inserted via the femoral artery. Coronary sinus was catheterized from the femoral vein. Blood samples were drawn from both the coronary sinus and peripheral (femoral) vein before and thirty minutes after PTCA, and from peripheral (cubital) vein 1, 2, 5 days, 1 and 6 months after PTCA. Hematocrit, plasma fibrinogen concentration, plasma viscosity, whole blood viscosity, and corrected whole blood viscosity were determined after each sampling. Besides the hemorheological variables, at thirteen patients the oxygen free radical associated parameters also were measured before and thirty minutes after PTCA, plus 1, 2, 5 days, and 1 month after the procedure as described previously.

Examination of hemorheological factors in patients with diabetes mellitus

30 patients with diabetes mellitus (mean age: 57 ± 12 years) were involved in the study and their data were compared to those of 68 healthy subjects (mean age: 34 ± 10 years). Plasma glucose concentration of patients was 12.2 ± 5.1 mmol/l and mean duration of their disease was 14.5 ± 9.4 years; all the patients suffered from diabetic retinopathy. They were treated with oral antidiabetic drug (12 patients) or insulin (18 patients). Hematocrit, plasma fibrinogen, plasma and whole blood viscosities, plus red blood cell aggregation parameters were measured. RBC aggregation was characterized by two methods: Myrenne MA-I aggregometer and LORCA aggregometer.

Results were presented as means ± SEM, and Students' t test was used to compare the values. All the patients and the control subjects of the studies gave an informed consent before blood sampling. Investigations were approved by the Regional Ethics Committee.

4. RESULTS

4.1 Comparative examination of red blood cell aggregation

Analyzing all of the samples as a function of plasma fibrinogen, a significant correlation was found with AI_L, nevertheless, correlation with AI_M or AI_{MI} could not be proved. Similar results turned up regarding plasma viscosity. There was not a significant correlation between AI_L and AI_M, whereas correlation of AI_L and AI_{MI} were significant. Further analysis based on the scatter plot diagram revealed that at fibrinogen concentration higher than 4.5 g/l there is no further increase in aggregation indices. Excluding samples with that high fibrinogen concentration, significant correlation could be calculated between AI_{MI}, AI_L and fibrinogen. Using the same data, correlation between AI_M, AI_{MI} and AI_L could also be found. Minimum disaggregation shear rate showed significant correlation with plasma fibrinogen concentration and plasma viscosity.

In this study measurements were carried out at the native hematocrit of the samples. Correlation between the results of Myrenne aggregometer and hematocrit could not be revealed, yet there was a correlation between LORCA aggregation index and hematocrit. We examined those samples in the 40-45 % hematocrit range separately – that is considered the optimal range for aggregation measurements in Myrenne aggregometer according to previous

studies - to see if there was a change in the relation of aggregation and plasma fibrinogen concentration. Correlation values regarding AI_M and AI_{M1} versus fibrinogen did not show any improvement in this sub-group, while correlation was even somewhat stronger regarding AI_L.

4.2 Hemorheological variables in coronary artery disease

Hematocrit level of IHD patients was significantly higher than that of controls ($p < 0.05$-0.01). Patients with multivessel disease had significantly elevated hematocrit comparing to the other IHD groups ($p < 0.05$-0.02). Plasma fibrinogen level of normals was significantly lower than that of patients ($p < 0.05$ between G-0 and G-1, $p < 0.01$ between G-0 and G-2, and between G-0 and G-3). Plasma fibrinogen also showed a significant elevation in the single and multivessel disease groups comparing to patients without significant coronary artery disease ($p < 0.05$). Plasma viscosity was increased in IHD patients ($p < 0.05$-0.001), and in G-2 and in G-3 higher values were found comparing to G-1 ($p < 0.05$ and 0.001). Whole blood viscosity was elevated in IHD patients ($p < 0.05$-0.01). The multivessel disease group had higher levels of WBV than single vessel disease subjects and patients without significant lesions ($p < 0.01$). The two latter groups did not differ from each other in this parameter. Among hemodynamic parameters, the cardiac index at rest did not differ statistically in normals and in IHD subjects, but CRI was significantly reduced in our patients ($p < 0.05$-0.01). In the multivessel disease group significantly lower values were found than in single vessel disease ($p < 0.05$).

4.3 Hemorheological changes during and after percutaneous transluminal coronary angioplasty

There was a significant decrease in the values of hematocrit, plasma and whole blood viscosity right after PTCA, while corrected whole blood viscosity remained unchanged during this period. Hematocrit level returned to the baseline during the days after PTCA and then an elevation at the end of the first and the sixth month could be observed. Plasma fibrinogen level elevated markedly during the days after PTCA with a peak value on the second day then returned to the baseline after one month. Another significant increase could be seen after six months. Plasma viscosity showed a similar trend to that of fibrinogen after PTCA, but seemed to remain higher than baseline during the following months. Whole blood viscosity - after the post-PTCA reduction - increased significantly by the end of the study period, while corrected

blood viscosity showed a continuously increasing tendency with a significant elevation on day 5 and afterwards.

4.4 Free radical mediated reactions during and after percutaneous transluminal coronary angioplasty

Blood TBARS concentration increased significantly ($p < 0.05$) in the first day and was elevated in the following days and returned to the baseline at the one month control visit. Catalase activity showed a reduction after PTCA followed by significantly increasing values on the days after PTCA and at the end of the first month ($p < 0.01$). Plasma SOD activity was elevated in the sample from the coronary sinus comparing to that from the peripheral vein before the procedure; 30 minutes after PTCA an increased level in the peripheral sample was also found ($p < 0.02$), and then returned to the baseline next day. Activity of SOD in blood was significantly reduced at the fifth day sampling ($p < 0.05$). There was a slight but significant decrease in the concentration of reduced glutathione of both samples 30 minutes after the intervention ($p < 0.02$) with the same concentration on the following day. Activity of glutathione peroxidase was significantly decreased in the first day, and then returned to the baseline. Its course showed a similar tendency to that of reduced glutathione. Superoxide production of leukocytes showed an increasing tendency ($p = 0.05$) referring to the possible over-activation of these cells one day after PTCA.

Spontaneous platelet aggregation was significantly higher in the samples from the coronary sinus than in those from the peripheral vein. Spontaneous platelet aggregation in the periphery and epinephrine-induced aggregation from both sites were markedly elevated after PTCA. On the following days platelet aggregability decreased to the baseline. Platelet aggregation induced by ADP or collagen showed slight but significant elevation in the samples from the coronary sinus.

4.5 Examination of hemorheological factors in patients with diabetes mellitus

Hematocrit, plasma fibrinogen concentration, plasma viscosity and whole blood viscosity at 90 s^{-1} were found significantly higher in patients. There was a tendency to higher values in aggregation indices measured by Myrenne aggregometer, although it was not significant. Aggregation index of patients determined by the LORCA was significantly higher

than that of healthy subjects. One of the dynamic parameters, $\gamma_{dise\text{-}min}$ that represents the minimum shear which is necessary to disrupt RBC aggregates was also significantly increased at patients; and $T_{1/2}$ that characterizes the speed of aggregate formation was markedly lower at diabetic persons.

5. SUMMARY

1. The comparison of Myrenne aggregometer and LORCA aggregometer was performed for the first time in the clinical practice. We could reveal that red blood cell aggregation in human blood is in correlation with plasma fibrinogen concentration if it is lower than a certain value, but there is no further increase at higher concentration, which is opposite to previous studies.

2. In our studies LORCA aggregometer was found to be better than Myrenne aggregometer giving more precise characterization of the red blood cell aggregation process. Our results emphasize the importance of standardization of measurement techniques.

3. We could show that hemorheological factors deteriorate in patients with more severe coronary artery disease, and this implies their pathophysiological role in the progression of this disease.

4. We could reveal significant alterations in hemorheological parameters after percutaneous transluminal coronary angioplasty. These changes along with those of the free radical associated parameters can have deleterious effect on the coronary circulation; it can also compromise the outcome of the procedure. We could reveal differences in some of the results of samples from the coronary sinus and the periphery; this finding calls the attention to that subtle changes could be hidden by dilution at peripheral samplings.

5. All the measured hemorheological variables were found to be elevated significantly or in tendency in patients with diabetes mellitus. Values of patients with non-proliferative and proliferative retinopathy did not differ from each other. This was the first study that used LORCA aggregometer to determine red blood cell aggregation in diabetes. Its results characterizing the aggregation process both quantitatively and dynamically showed markedly worse values of patients.

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