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. In spite of the extended research on this field, there still remained obscure parts in the pathomechanism of these diseases. Investigations explored the role of vascular wall, cellular interactions, and many of the underlying biochemical processes [13,17]. Much less attention was paid to the properties of the circulating blood. Therefore our first purpose was to investigate the hemorheological characteristics of patients with cardiovascular diseases.

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 (a suspension of cells, chylomicrons, carbohydrates, proteins, electrolytes in an aqueous medium) and its viscosity – unlike to that of Newtonian fluids - 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 (hematocrit) to the viscosity has been demonstrated in numerous studies. Besides hematocrit, the size, shape and internal viscosity of the red cells can affect the 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 [44,61,62].

Interactions between cellular components of blood such as aggregation, agglutination or adhesion of cells are observed in a variety of normal and pathological conditions. Rouleaux formation of red blood cells in static blood, platelet adhesion and aggregation in hemostatic plug formation and thrombosis or leukocyte adhesion to the endothelium are examples of such interactions. In the case of red blood cells the agglutination is caused by specific binding of immunoglobulins, while the aggregation of the cells (rouleaux formation) is caused by non-specific action of fibrinogen and some other big molecules of blood plasma (Figure 1.1). 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 (on the contrary, agglutination is completely irreversible) [5,11].

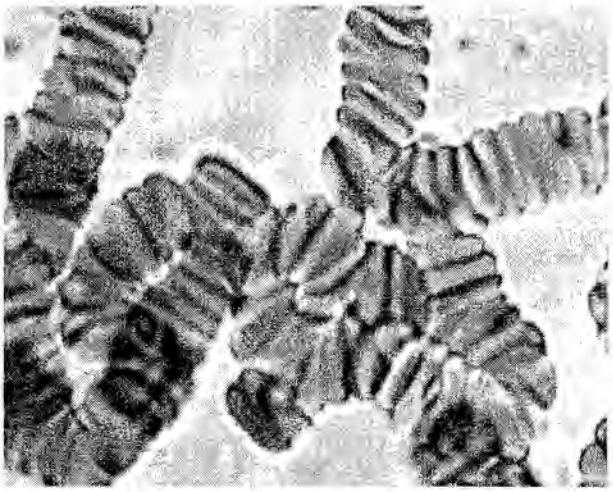

Figure 1.1 Red blood cells illustrating rouleaux formation.

There exist two hypotheses explaining the mechanism of red blood cell aggregation process. The bridging hypothesis implies molecular bridges between adjacent cells. According to this, unspecifically adsorbed molecules like fibrinogen and large molecular weight globulins in blood or dextran in RBC suspension create bridges between the surfaces of 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) [5,67].

Red blood cell aggregation is a dynamic process that is also affected by shear stress, membrane properties, and cell deformability. The latter two features have an important impact on the maximum contact area between the aggregating cells. 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 [11].

In most cases ischemic heart disease is 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 [18,69]. In several studies hemorheological parameters were proved to be primary cardiovascular risk factors [18,52,68]. The

Framingham Study, Puerto Rico Heart Health Program and Honolulu Heart Program proved hematocrit to be a cardiovascular risk factor [12,31]. Framingham Study and Northwick Park Study proved the same for elevated plasma fibrinogen concentration. The Monica Project showed viscosity as a risk factor [40]. Edinburgh Artery Study and Caerphilly and Speedwell Collaborative Heart Disease Study provided further confirmation of the role of several hemorheological variables in cardiovascular diseases [37,41,63,72]. In the Physicians' Health Study the elevated baseline plasma fibrinogen concentration was associated with the increased risk of future myocardial infarction during a five-year follow-up period and this was independent from other risk factors [42].

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 (the diameter can be as small as 3-5 μm, several micrometers smaller comparing to that of RBCs (Figure 1.2)). Therefore the role of rheological alterations may be of higher importance than in other parts of the circulatory system [3,32,68]. Lowe et al. suggested that blood viscosity is related to the extension of coronary heart disease, but they did not find such a relation regarding plasma viscosity [38]. Rainer et al. could find worse hemorheological variables in patients with angiographically proven coronary artery disease, but there was not any relation with the extent of the disease [56]. However, these studies involved only a small group 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.

Figure 1.2 Deformation of a red blood cell in a pore with lower diameter than that of the cell.

Percutaneous transluminal coronary angioplasty (PTCA) 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 [39]. 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 [26,50]. 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, glutathion peroxidase) and several molecules, among these thiol-group containing substances, e.g. glutathion. Oxidative stress following ischemia and reperfusion of the myocardium results in the generation of free radicals in the endothelial, the myocardial and the blood cells that have a great impact on cellular characteristics and interactions, hemorheological conditions, and tissue damage. Due to the high reactivity, free radicals have very short lifetime, thus their generation can mainly be detected indirectly measuring the end products of free radical mediated reactions (e.g. the concentration of malondialdehyde as a marker of lipid peroxidation) or the activity of the antioxidant systems (Figure 1.3).

$$2\,O_2^{\bullet -} + H_2 \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

$$2\,H_2O_2 \xrightarrow{\text{CATALASE}} 2\,H_2O + O_2$$

$$H_2O_2 + 2\,GSH \xrightarrow{\text{GPX}} 2\,H_2O + GSSG$$

Figure 1.3 Reactions catalyzed by the antioxidant enzymes.

Free radical mediated processes have been investigated in various animal models and some clinical conditions. Elevated free radical activity was reported in patients with coronary artery disease without predicting value on the severity of the disease [10]. 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. Our second goal was to explore the free radical associated changes during and after percutaneous transluminal coronary angioplasty.

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 (which can involve the adhesion of these cells to the vascular wall) 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 (Figure 1.4). 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.

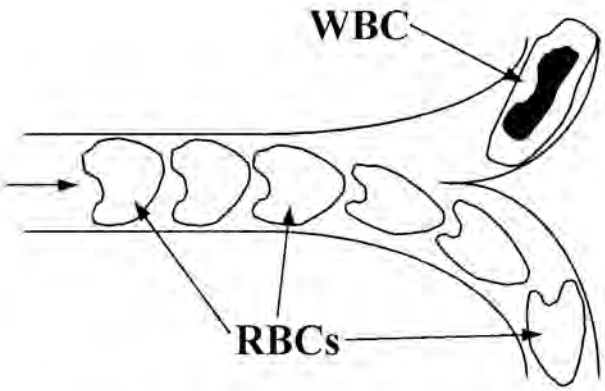

Figure 1.4 Entry of a white blood cell into a capillary branch can make all red blood cells enter the other branch.

This impairment does not necessarily take place only during ischemia, but it can be a causal factor in "no-reflow" phenomenon. This phenomenon implies that even after the restoration of blood flow in a larger artery branch there still remain areas of the tissue without

sufficient circulation due to the incomplete recovery of capillary flow. Besides the swelling of the endothelial and tissue cells, white blood cell plugging, formation of red blood cell aggregates and platelet aggregates can be involved in this process, which might be reversed by a hemorheologic therapy.

Diabetes mellitus is a disease of high social and economic importance. It 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. In the development of these complications several metabolic disturbances are involved: 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). It is also conceivable that hemorheological factors become even more important, when vessel radius, the major determinant of blood flow has already been reducted which is common finding in diabetes [2].

Microvascular damages affect the vessels of the retina, the myocardium, the kidney, the skin and the nervous system more severely. In the eye thickening of the basal membrane of capillaries occurs and the chemical composition of the membrane is altered, so it becomes more permeable to a variety of substances; endothelial cells also become abnormal. One of the main characteristics of vasculopathy is the reduction of perfusion in capillaries, even if bigger vessels have normal (or increased) perfusion due to arteriovenous shunt flow. This capillary occlusion results in retinal hypoxia and tissue damage, and as a consequence can lead to the phenomenon of neovascular formation [53].

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. Most of the studies found red blood cell aggregation elevated. In general, the pathophysiological role of hemorheological factors in diabetes mellitus is proposed, but probably because of the great variety of the study populations and the techniques used in the different investigations results were not unequivocal, and therefore further evidence is necessary to support the previous findings [2].

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. [43]. 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:

$$\frac{WBV_{40\%}}{PV} = \left[\frac{WBV_{HCT}}{PV}\right]^{\frac{40\%}{HCT}}$$

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 [15].

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 (Figure 3.1).

Figure 3.1 Myrenne MA-1 aggregometer.

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 (Figure 3.2). Measurements are carried out at room temperature (22 ± 1 °C) [4,21,44].

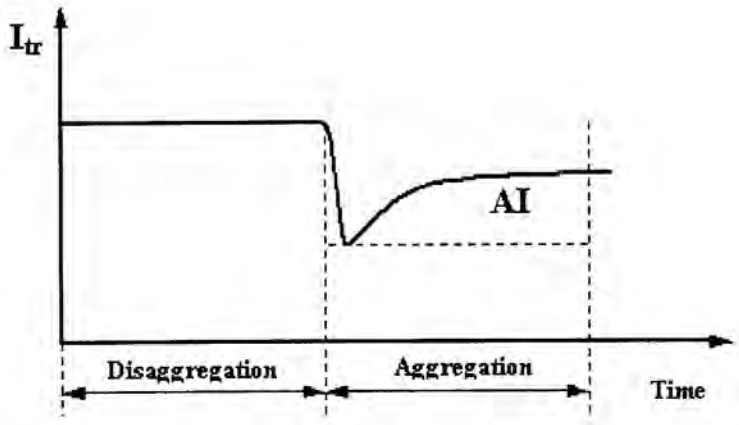

Figure 3.2 Light intensity changes during the measurement in Myrenne aggregometer. Intensity of light transmission (I_{tr}) is plotted as a function of time. After disaggregation at high shear rate, the stop of shearing results in a sudden drop of light transmission due to the loss of orientation and elongation of cells; and then light intensity increases as more and more cells become aggregated. Aggregation index is calculated as the area under the curve in the first 10 seconds of the aggregation process.

In the recently developed Laser-assisted Optical Rotational Cell Analyzer (Figure 3.3) 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 (Figure 3.4) [27,28].

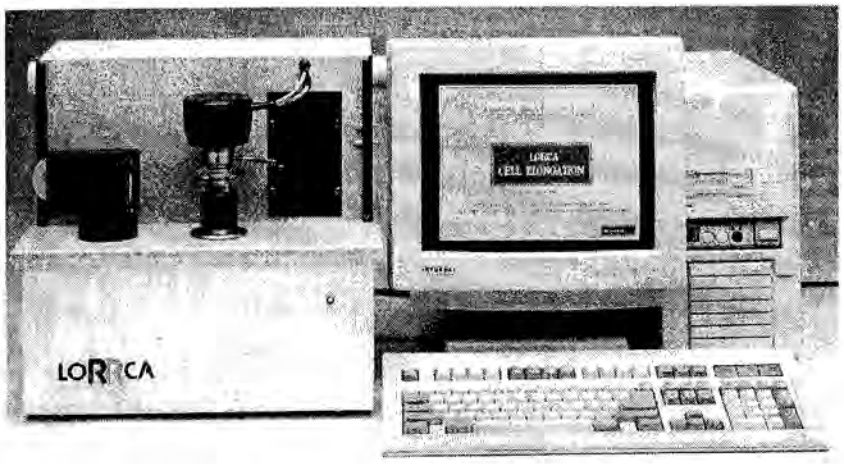

Figure 3.3 Laser-assisted Optical Rotational Cell Analyzer (LORCA). Basic instrument (left), and computer system (right).

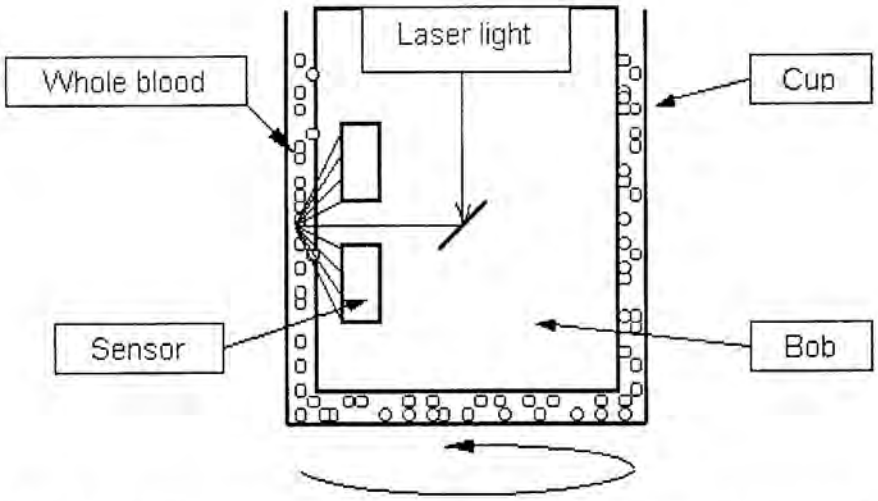

Figure 3.4 Schematic drawing of LORCA aggregation measurement.

Aggregation index is calculated as a ratio of the area under 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 (Figure 3.5).

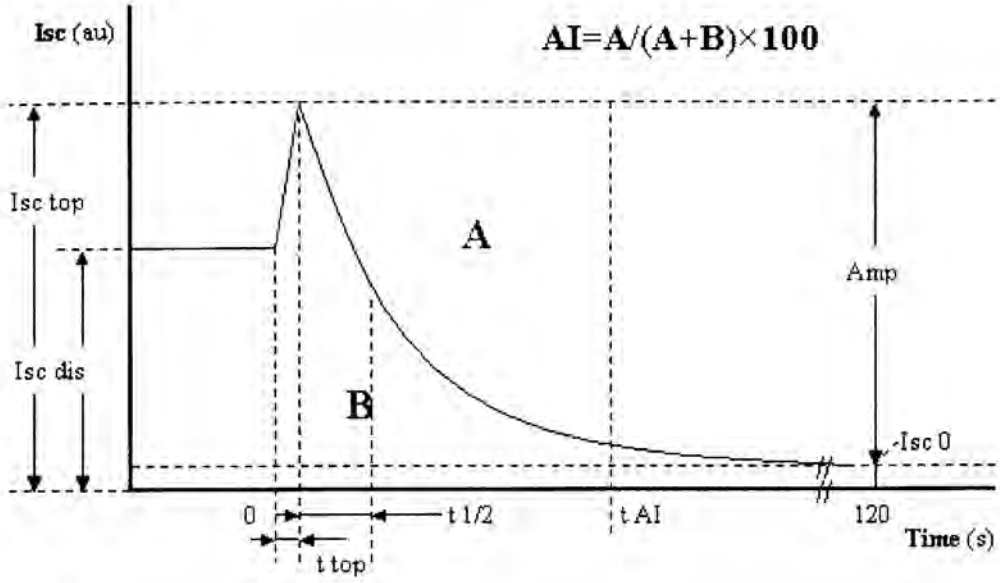

Figure 3.5 Schematic drawing of the syllectogram (aggregogram) showing the light intensity changes during the RBC aggregation process in LORCA system. I_{sc}: intensity of light backscattering (in arbitrary units (au)); $I_{sc\ dis}$: intensity at disaggregation; $I_{sc\ top}$: maximum intensity after the stop of shearing due to the loss orientation and elongation of RBCs; $I_{sc\ 0}$: minimum intensity at maximal aggregation; Amp: $I_{sc\ top}$-$I_{sc\ 0}$; $t_{1/2}$: time to half Amp; A: area above the curve; B: area under the curve.

With LORCA aggregometer it is also possible to determine the minimum disaggregation shear rate ($\gamma_{disc\text{-}min}$) 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. (Figure 3.6).

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. Correlation coefficients were calculated by SPSS statistical computer program.

Aggregation		Disaggregation		
Shear (1/s)	Isc (au)	Shear (1/s)	Isc (au)	dIsc (au)
10	17.41	400	22.58	**5.17**
200	23.68	400	22.60	**-1.08**
400	22.55	400	22.60	**0.05**
100	23.81	400	22.56	**-1.25**
20	19.19	400	22.56	**3.37**
800	21.66	400	22.64	**0.98**
60	22.45	400	22.63	**0.17**
80	23.65	400	22.67	**-0.98**
150	24.41	400	22.76	**-1.65**
125	24.27	400	22.76	**-1.51**
175	23.94	400	22.70	**-1.25**

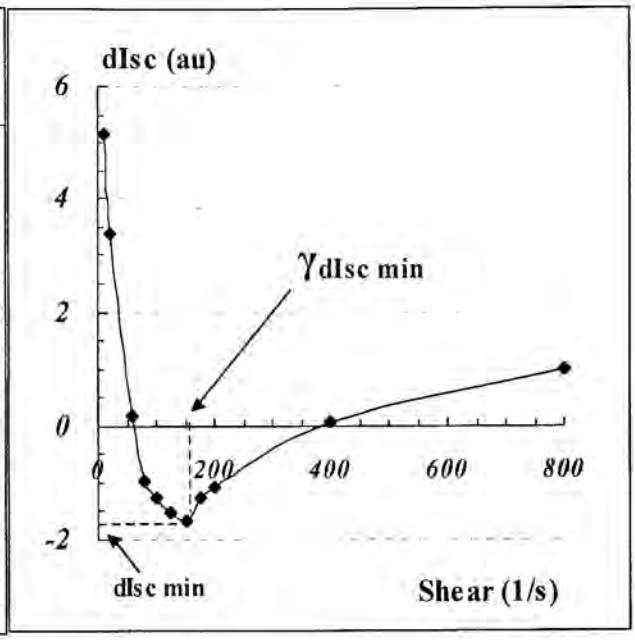

Figure 3.6 An example of determination of the minimum disaggregation shear rate.

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 [54].

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 [45]. 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) [7]. Glutathione peroxidase activity of red blood cells was measured by the Chiu-Stultz method [14]. Concentration of reduced glutathione of blood was measured using Ellman's technique [19,59].

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 [24].

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:

$$\text{Circulatory index } (l/N/s^2) = \frac{\text{Cardiac index } (ml/s/m^2)}{\text{Whole blood viscosity at } 90\ s^{-1}\ (mPas = mNs/m^2)}$$

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_{MI} 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.

Table 3.1 Characteristics of patients undergoing coronary angiography (data are presented as mean ± S.D. at age, mean ± S.E.M. at serum lipids, body mass index, cardiac index and ejection fraction).

	Whole population	Group 1	Group 2	Group 3
Number of patients	162	31	29	102
Age (years)	55 ± 10	51 ± 10	51 ± 10	57 ± 10
Sex (%) male female	64 36	42 58	59 41	75 25
Previous myocardial infarction (%)	52	19	52	62
Hypertension (%)	67	71	55	70
Diabetes mellitus (%)	21	6	21	28
Smoking (%) never previously currently	53 17 30	81 4 15	36 16 48	49 22 29
Total cholesterol (mmol/l)	5.74 ± 0.13	5.61 ± 0.36	6.27 ± 0.28	5.72 ± 0.12
HDL cholesterol (mmol/l)	1.09 ± 0.04	1.19 ± 0.07	1.20 ± 0.06	1.07 ± 0.07
Triglycerides (mmol/l)	2.33 ± 0.14	2.12 ± 0.25	2.38 ± 0.22	2.53 ± 0.18
Body mass index (kg/m^2)	27.5 ± 0.4	27.6 ± 0.6	27.4 ± 0.7	27.8 ± 0.4
Cardiac index ($l/min/m^2$)	2.52 ± 0.06	2.51 ± 0.19	2.63 ± 0.16	2.57 ± 0.06
Ejection fraction (%)	52 ± 1	54 ± 2	53 ± 2	50 ± 1

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-1 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_{M1} 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_{M1} were significant (Table 4.1, Figures 4.1-4.3).

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 (Figure 4.1). Excluding samples with that high fibrinogen concentration, significant correlation could be calculated between AI_{M1}, AI_L and fibrinogen. Using the same data, correlation between AI_M, AI_{M1} and AI_L could also be found (Table 4.1, Figures 4.1-4.2).

Parameters characterizing the dynamics of aggregation measured by LORCA are presented in figures 4.4-4.5. Minimum disaggregation shear rate showed significant correlation with plasma fibrinogen concentration and plasma viscosity (Figure 4.4).

Table 4.1 Correlation coefficients: 1. data of the whole group; 2. data of the subgroup with fibrinogen concentration < 4.5 g/l.

	1. AI_L	AI_M	AI_{M1}	2. AI_L	AI_M	AI_{M1}
Plasma fibrinogen	0.578***	-0.187	0.266	0.495***	0.279	0.350*
Plasma viscosity	0.612***	-0.003	0.303*	0.570***	0.195	0.309*

	1. AI_L	2. AI_L
AI_M	-0.119	0.297*
AI_{M1}	0.385**	0.379**

*p < 0.05 **p < 0.01 ***p< 0.001

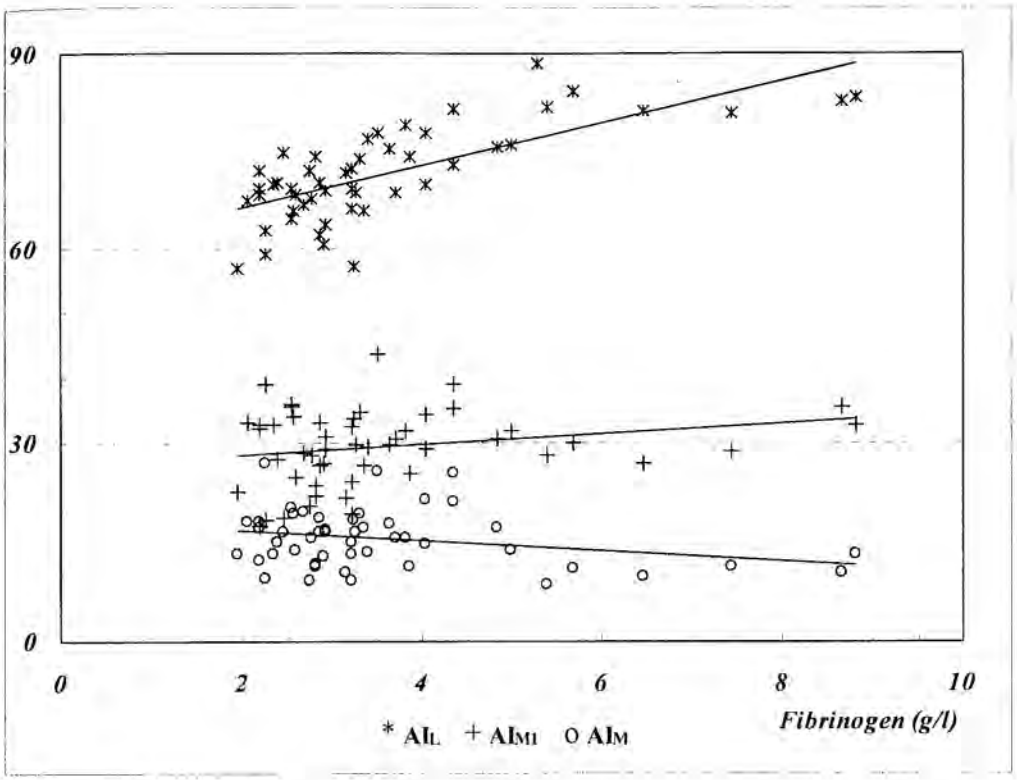

Figure 4.1 Aggregation indices plotted against plasma fibrinogen concentration. The correlation of AI_L and fibrinogen was significant ($p < 0.001$), whereas that of AI_{M1} and fibrinogen was significant only at lower concentration ($p < 0.05$); regarding AI_M, there was not any correlation.

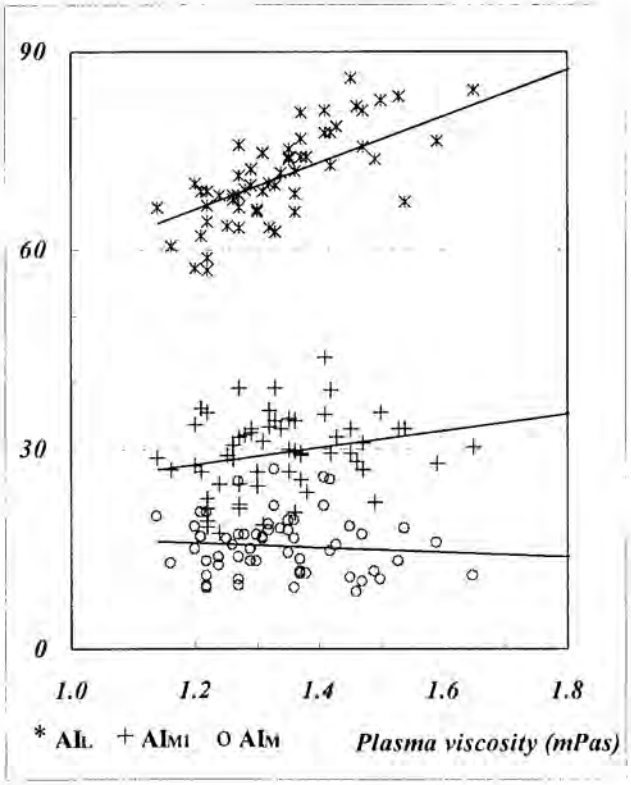

Figure 4.2 Aggregation indices as a function of plasma viscosity.

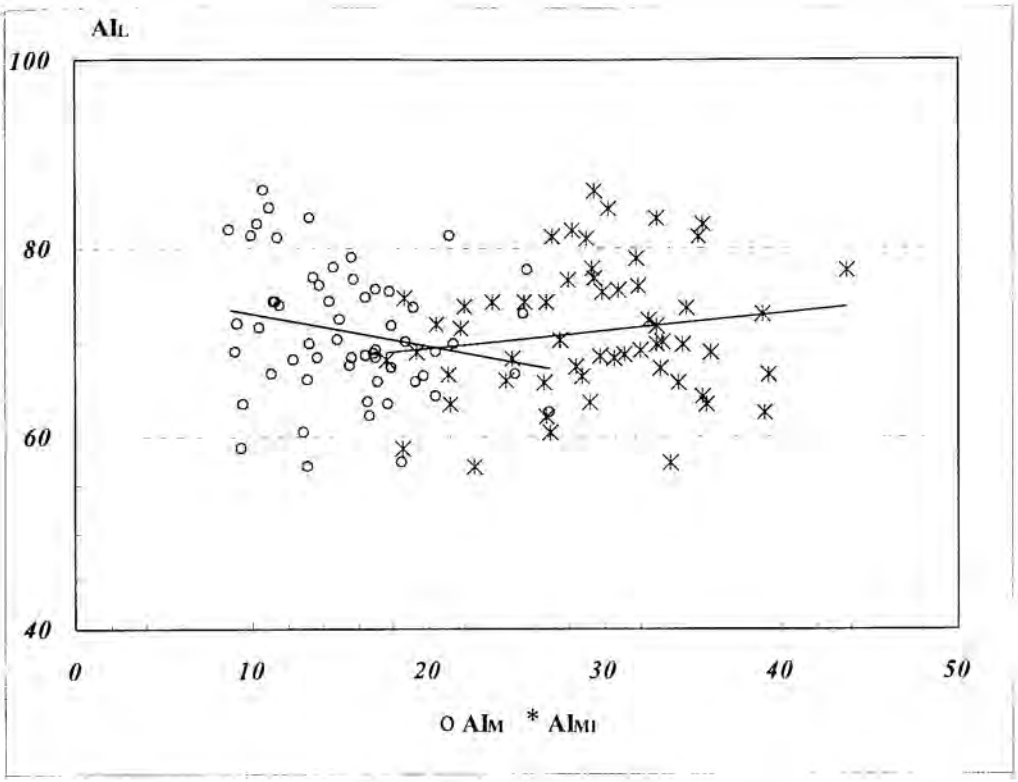

Figure 4.3 AI_L values are plotted against AI_M and AI_{MI}, respectively.

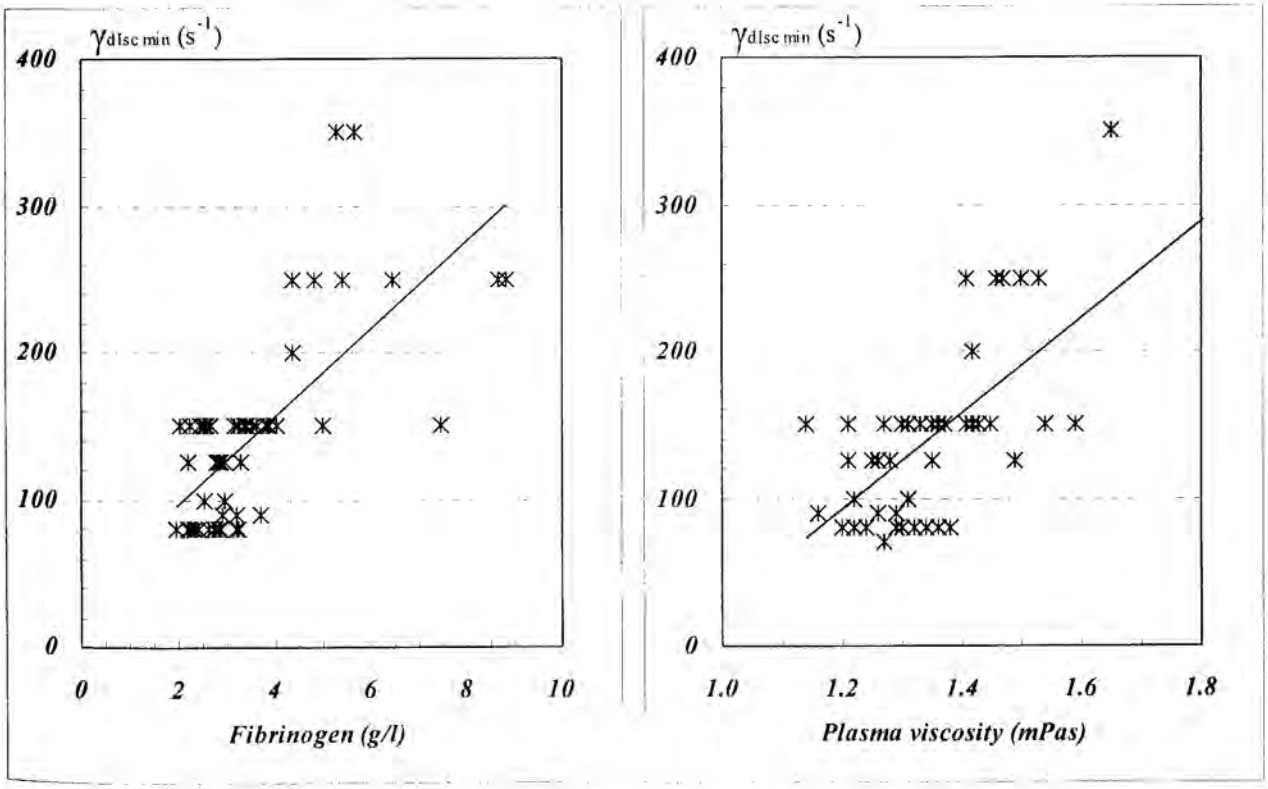

Figure 4.4 Minimal disaggregation shear rate ($\gamma_{dlsc\text{-}min}$) as a function of plasma fibrinogen concentration and plasma viscosity ('r' values: 0.687 and 0.739, respectively, $p < 0.01$).

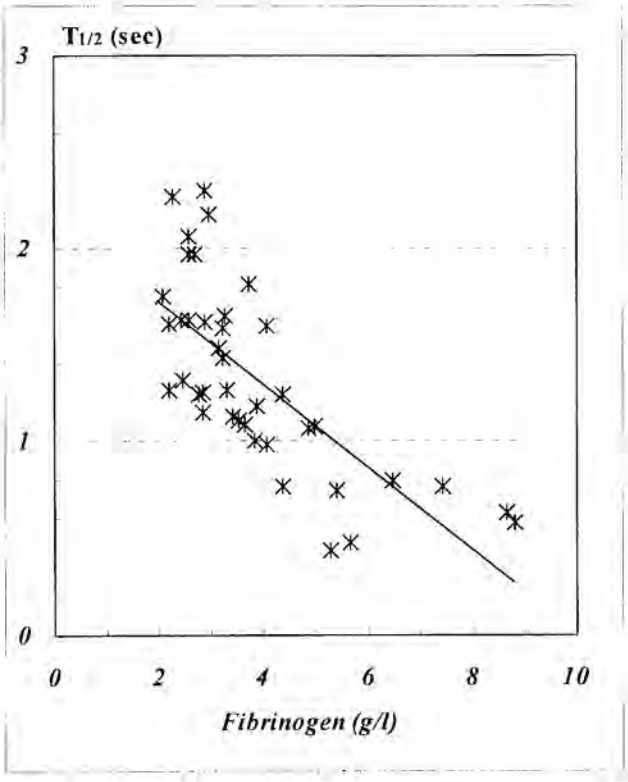

Figure 4.5 Time to the half of maximal aggregation vs. plasma fibrinogen concentration ('r' value: -0.35, $p < 0.05$). Higher fibrinogen concentration results in faster aggregate formation.

In figure 4.6 aggregation values are plotted against hematocrit. 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_{MI} versus fibrinogen did not show any improvement in this sub-group, while correlation was even somewhat stronger regarding AI_L.

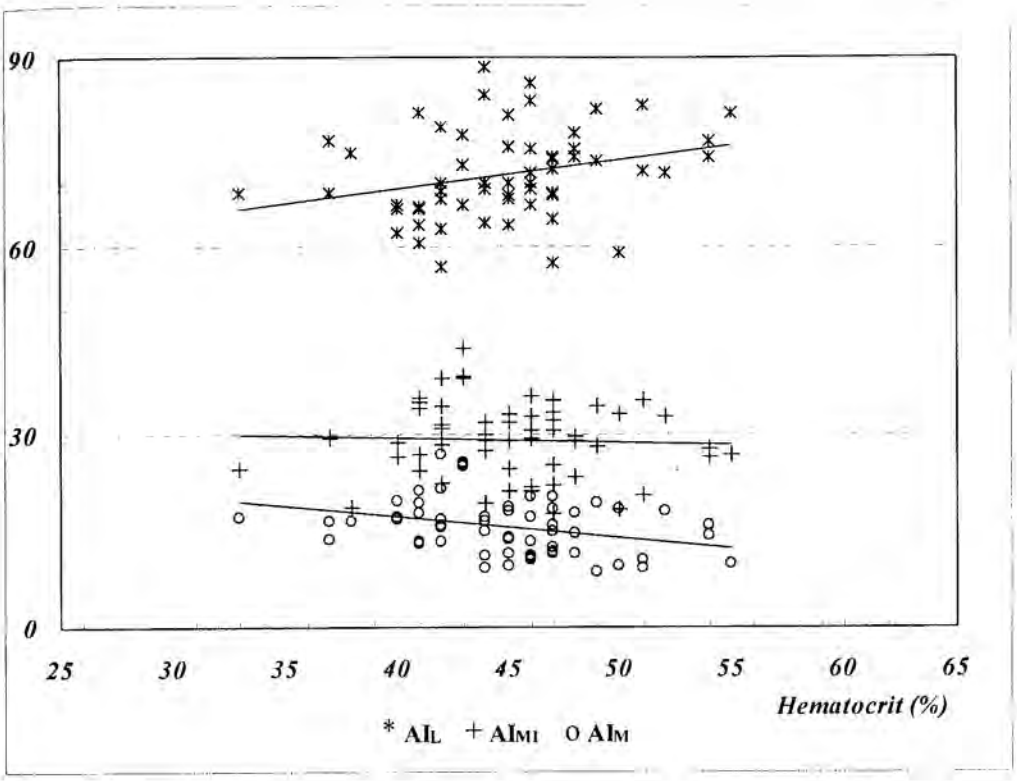

Figure 4.6 Values of aggregation indices are plotted against sample hematocrit.

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) (Figure 4.7).

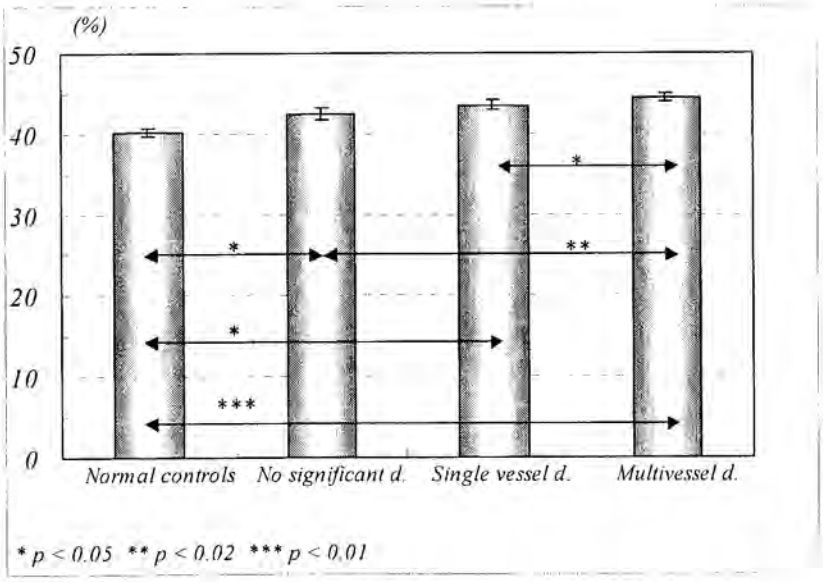

Figure 4.7 Hematocrit.

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 ($p < 0.05$) (Figure 4.8).

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) (Figure 4.9).

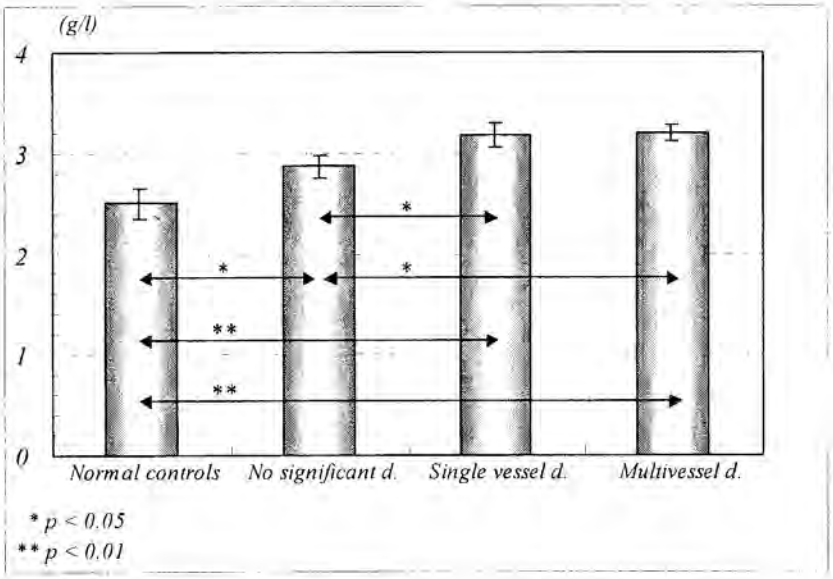

Figure 4.8 Plasma fibrinogen concentration.

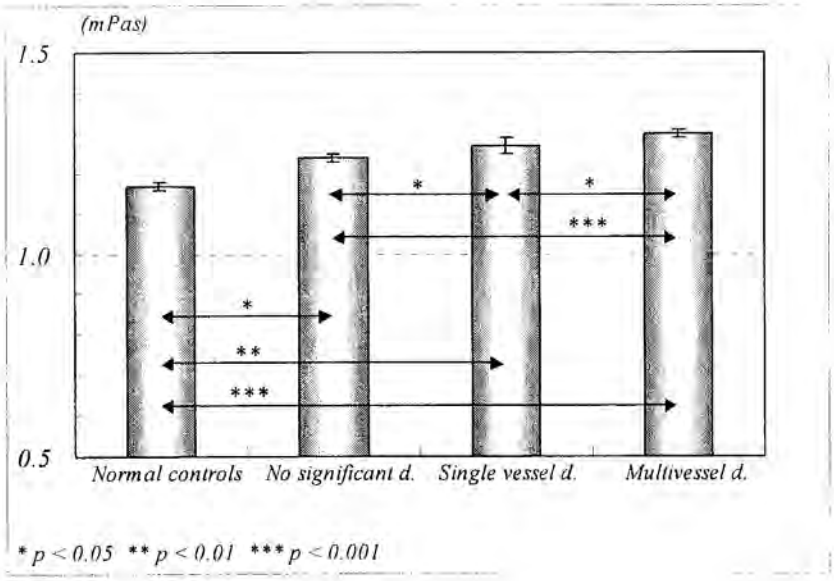

Figure 4.9 Plasma viscosity.

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 (Figure 4.10).

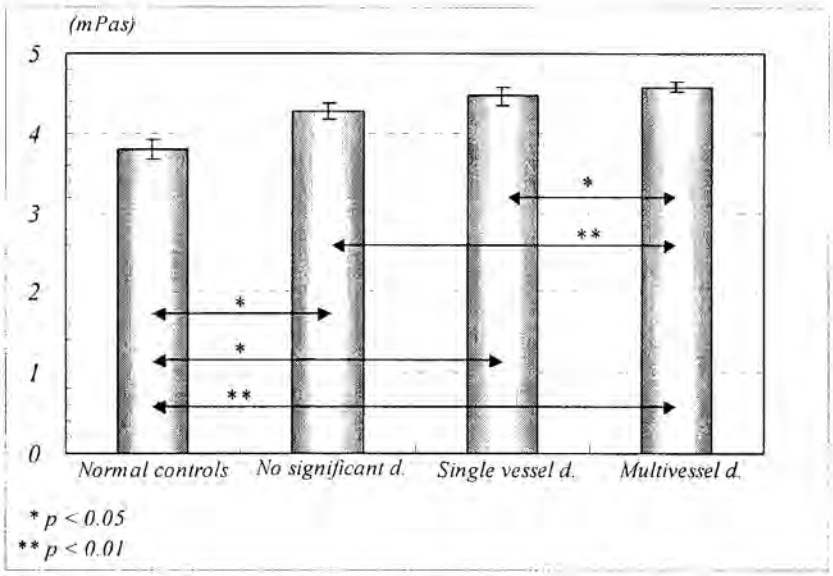

Figure 4.10 Apparent whole blood viscosity at 90 s^{-1} shear rate.

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 ($p < 0.05$) (Figure 4.11).

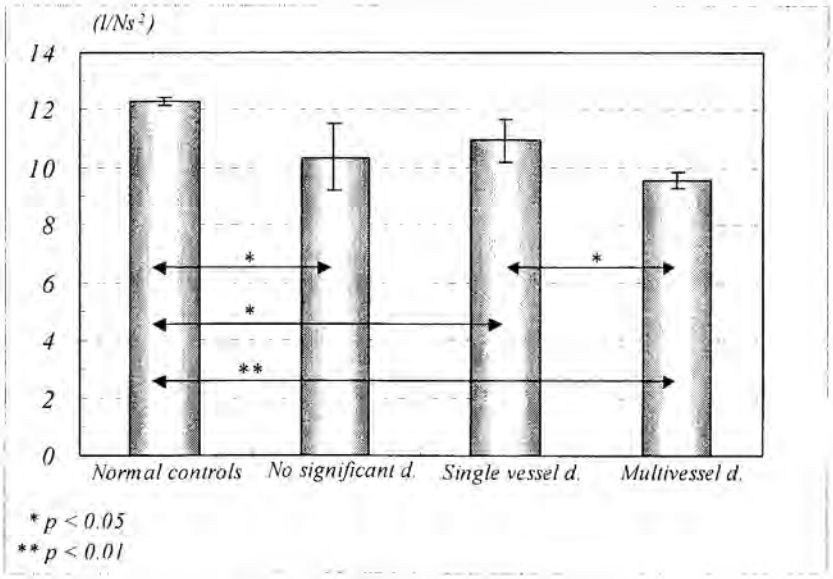

Figure 4.11 Circulatory index.

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 (Table 4.2).

Table 4.2 Hemorheological parameters before and 30 minutes after PTCA.

	Before PTCA		30 minutes after PTCA	
	Vein	Cor. sinus	Vein	Cor. sinus
Hematocrit (%)	40.6 ± 0.8	40.6 ± 0.8	38.3 ± 1.0 ***	38.6 ± 0.9 ***
Plasma viscosity (mPas)	1.20 ± 0.02	1.20 ± 0.02	1.18 ± 0.02 *	1.16 ± 0.02 **
Whole blood viscosity at 90 s^{-1} (mPas)	3.88 ± 0.13	3.98 ± 0.13	3.68 ± 0.13 *	3.78 ± 0.15 **
Corrected whole blood viscosity (mPas)	3.76 ± 0.08	3.86 ± 0.07	3.82 ± 0.05	3.90 ± 0.13

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

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 (Figure 4.12).

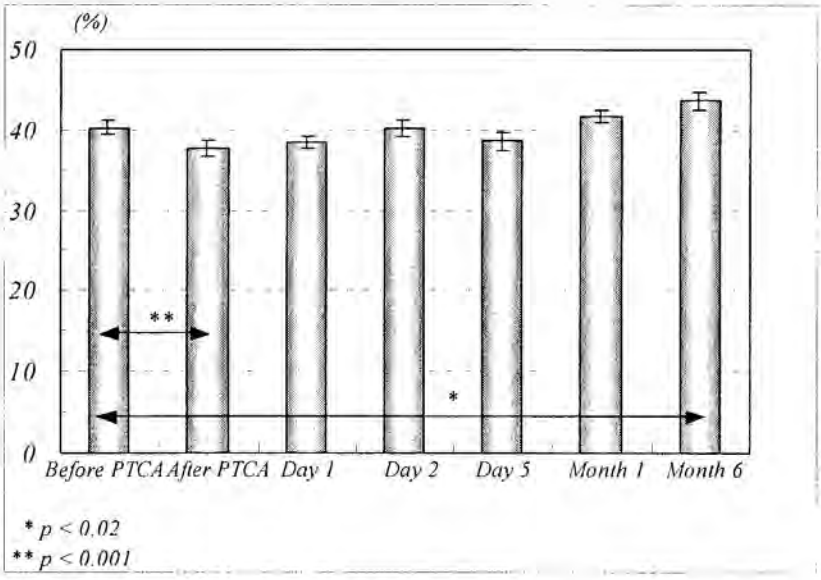

Figure 4.12 Hematocrit.

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 (Figure 4.13). Plasma viscosity showed a similar trend to that of fibrinogen after PTCA, but seemed to remain higher than baseline during the following months (Figure 4.14).

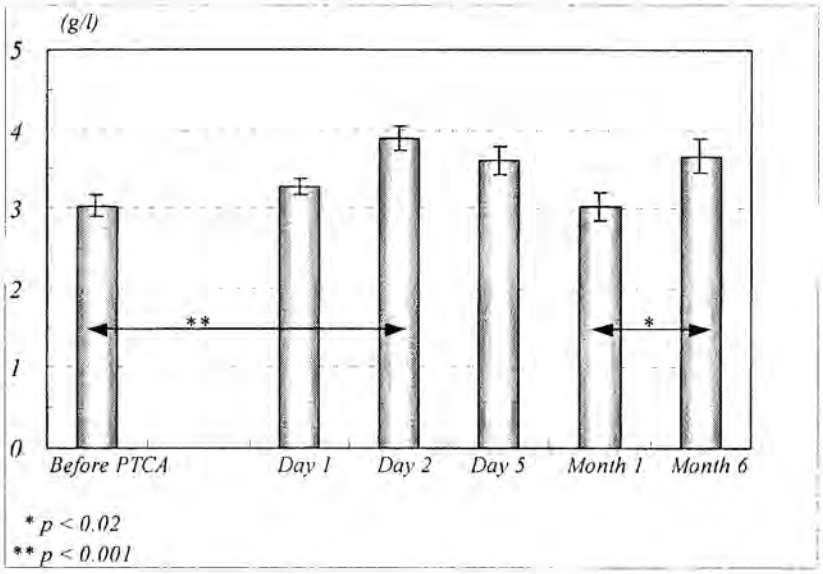

Figure 4.13 Plasma fibrinogen concentration.

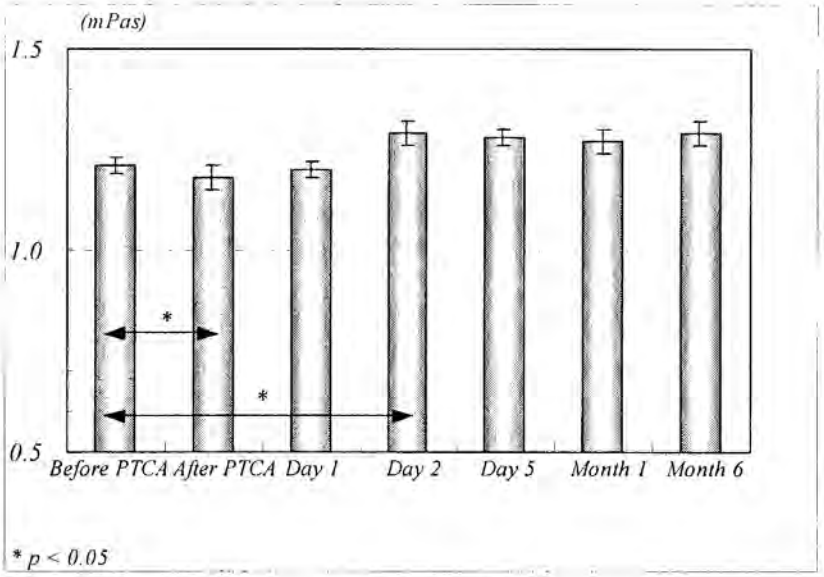

Figure 4.14 Plasma viscosity.

Whole blood viscosity - after the post-PTCA reduction - increased significantly by the end of the study period (Figure 4.15), while corrected blood viscosity showed a continuously increasing tendency with a significant elevation on day 5 and afterwards. (Figure 4.16).

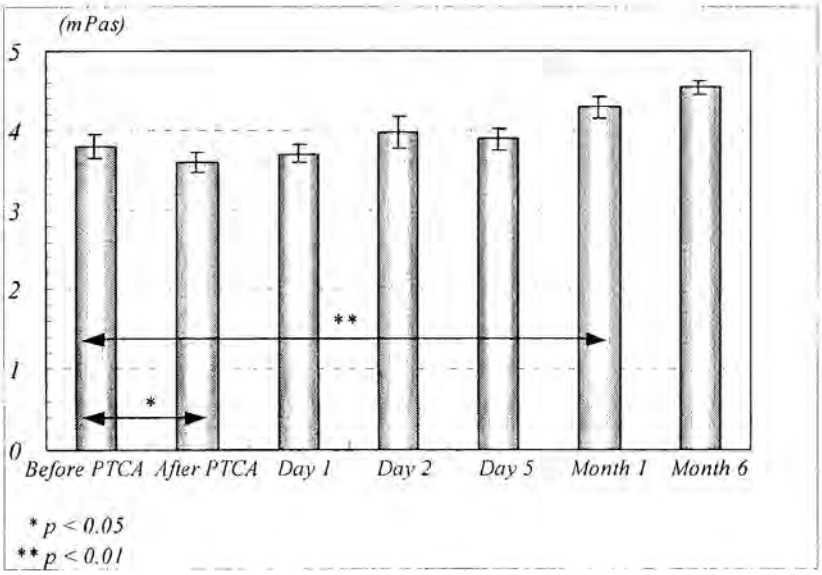

Figure 4.15 Apparent whole blood viscosity at 90 s^{-1} shear rate.

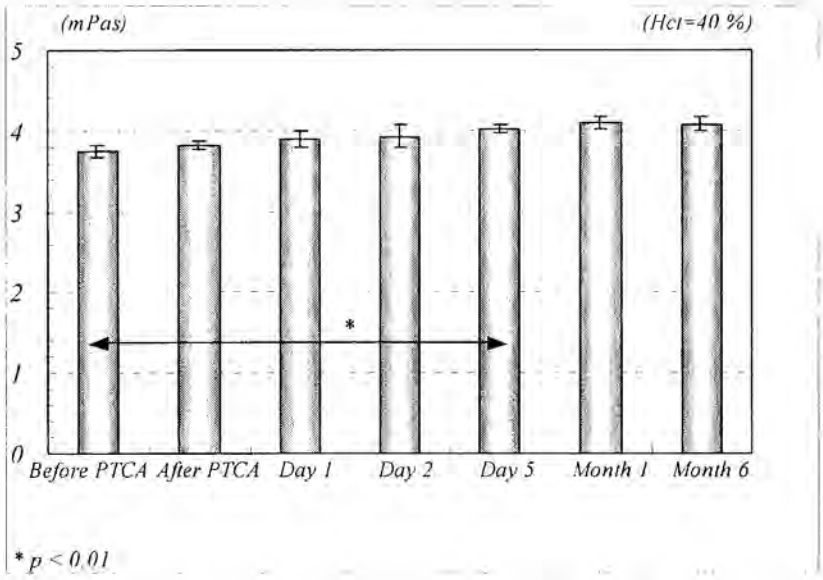

Figure 4.16 Apparent whole blood viscosity at 90 s^{-1} shear rate corrected to 40% hematocrit.

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 (Figure 4.17).

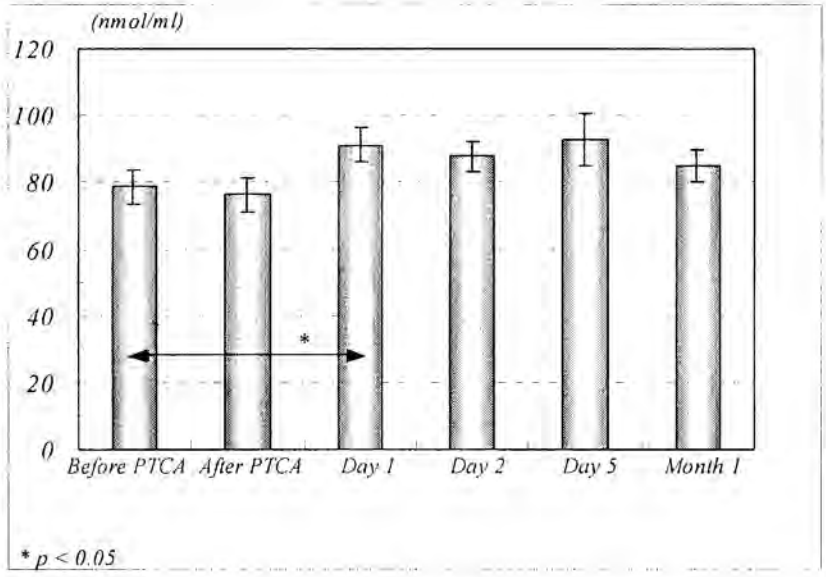

Figure 4.17 Blood TBARS concentration.

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 ($p < 0.01$) (Figure 4.18).

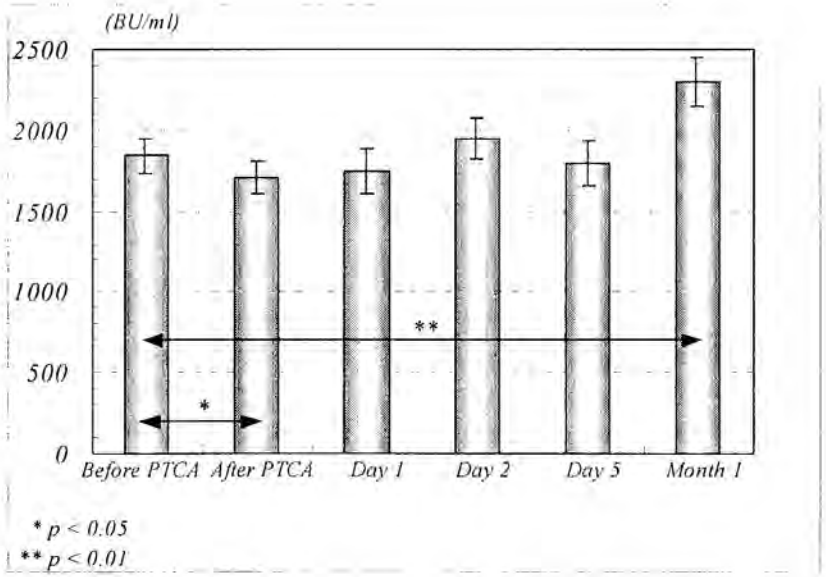

Figure 4.18 Activity of catalase of the red blood cells expressed in Bergmeyer units (BU/ml).

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 (Figures 4.19-4.20). Activity of SOD in blood was significantly reduced at the fifth day sampling ($p < 0.05$) (Figure 4.21).

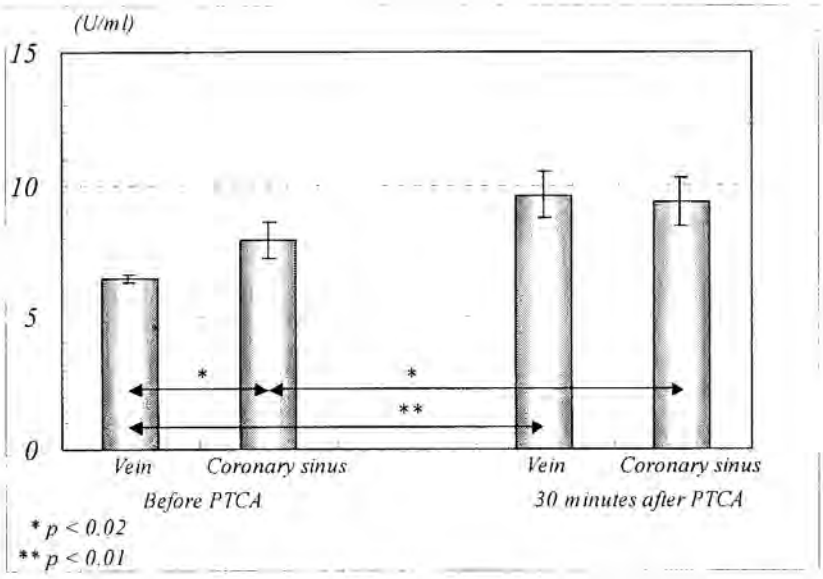

Figure 4.19 Activity of superoxide dismutase in the plasma.

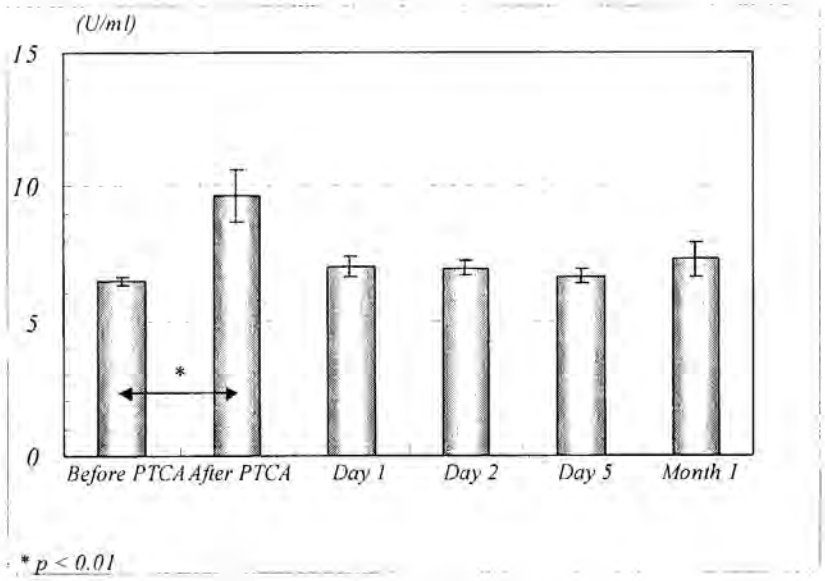

Figure 4.20 Activity of superoxide dismutase in the plasma.

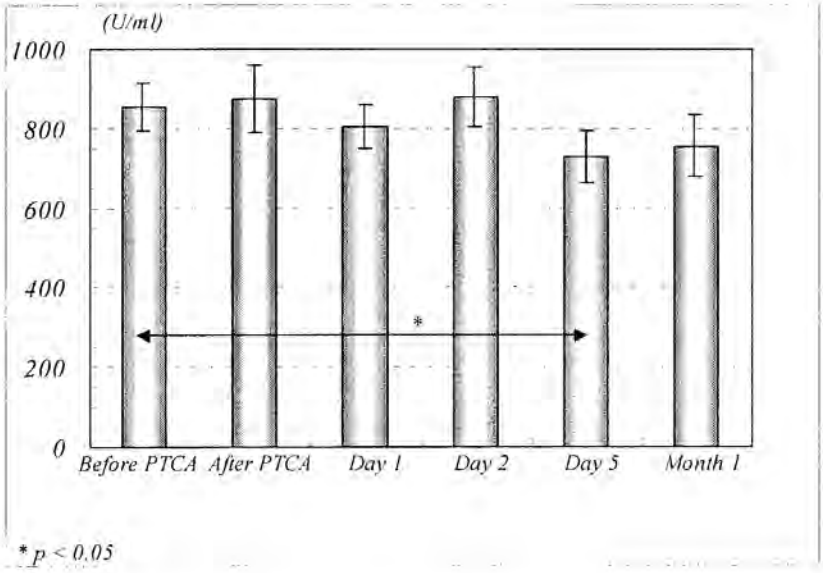

Figure 4.21 Activity of superoxide dismutase in the red blood cells.

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 (Figures 4.22-4.23).

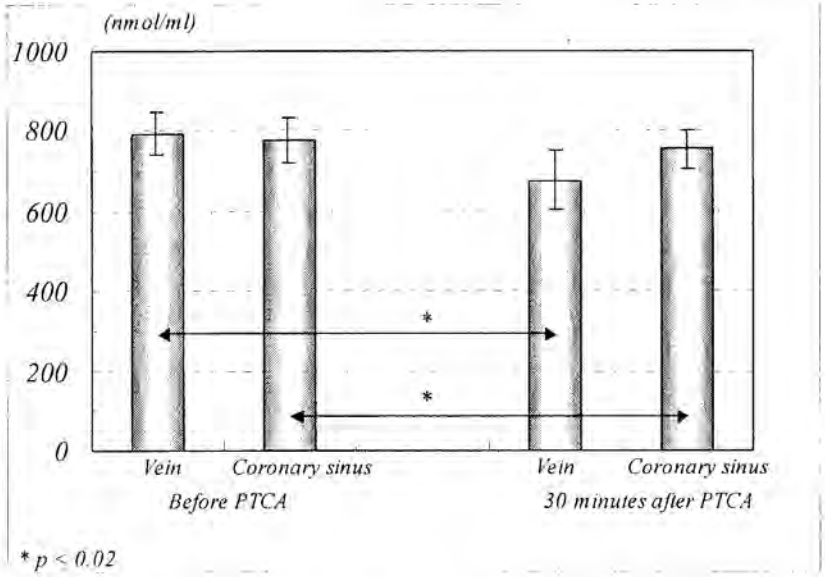

Figure 4.22 Concentration of reduced glutathione of blood.

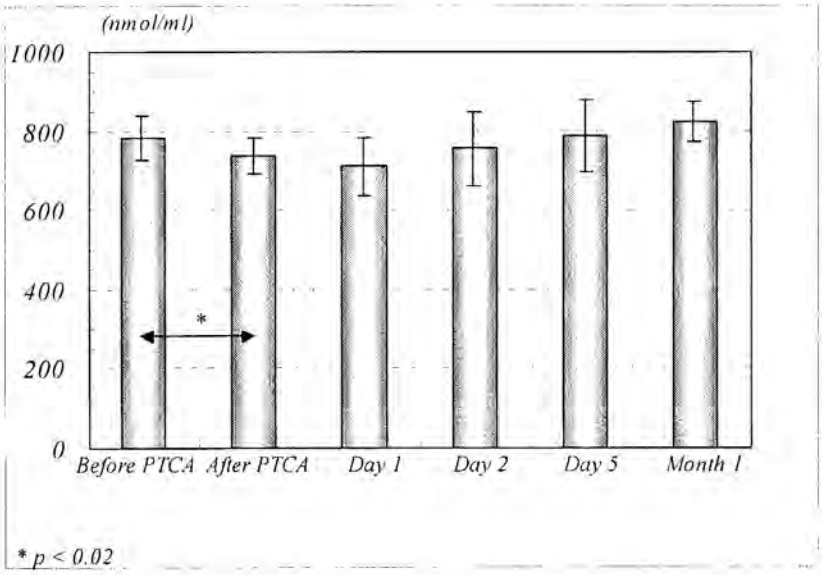

Figure 4.23 Concentration of reduced glutathione of blood before and after the intervention.

Activity of glutathione peroxidase was significantly decreased in the first day, and then returned to the baseline (Figure 4.24). Its course showed a similar tendency to that of reduced glutathione.

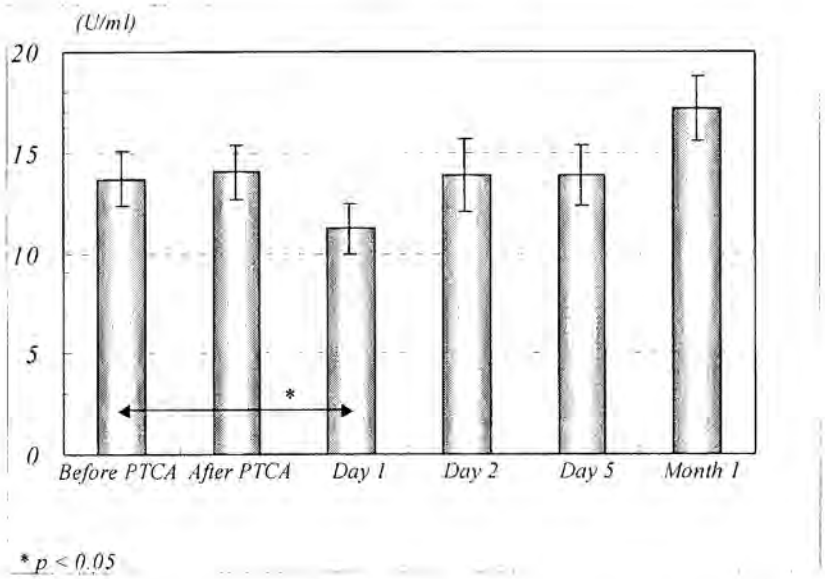

Figure 4.24 Activity of glutathione peroxidase in blood.

Superoxide production of leukocytes showed an increasing tendency ($p = 0.05$) referring to the possible over-activation of these cells one day after PTCA (Figure 4.25).

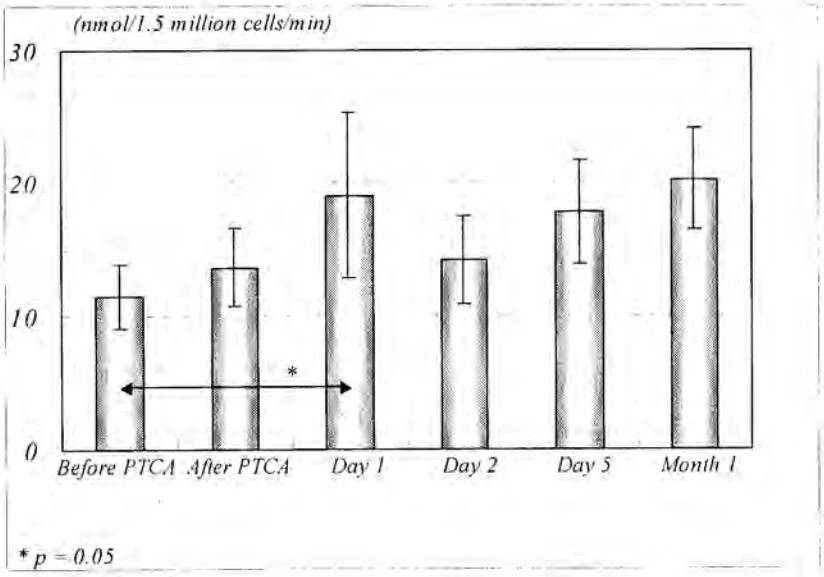

Figure 4.25 Superoxide generating capacity of the polymorphonuclear leukocytes.

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 (Figure 4.26).

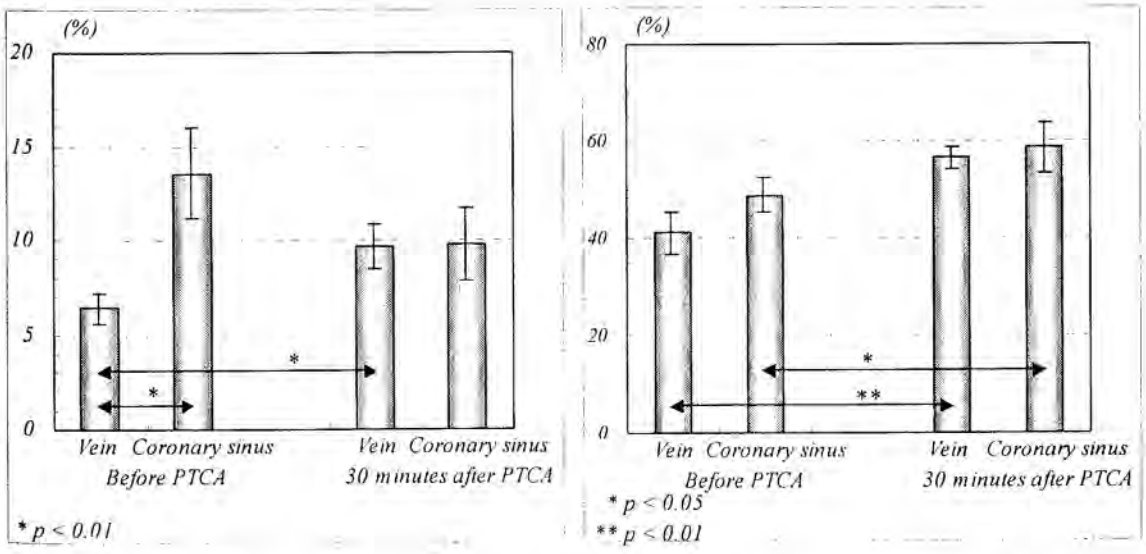

Figure 4.26 Spontaneous and epinephrine-induced platelet aggregation before and thirty minutes after PTCA.

On the following days platelet aggregability decreased to the baseline (Figure 4.27). Platelet aggregation induced by ADP or collagen showed slight but significant elevation in the samples from the coronary sinus.

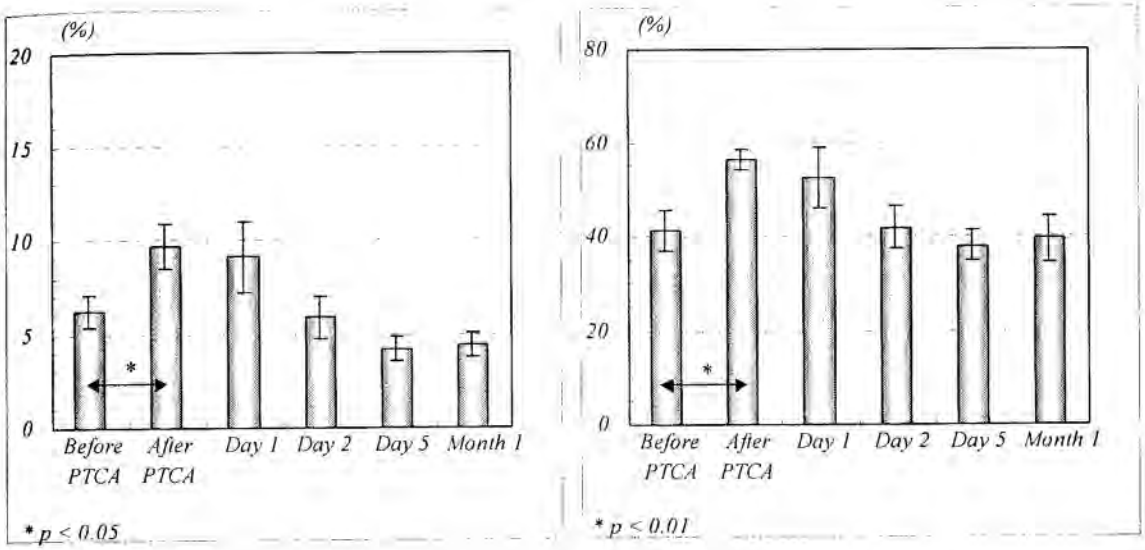

Figure 4.27 Spontaneous and epinephrine-induced platelet aggregation during the days and one month after PTCA.

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 (Figure 4.28). There was a tendency to higher values in aggregation indices measured by Myrenne aggregometer, although it was not significant (Figure 4.29). Aggregation index of patients determined by the LORCA was significantly higher than that of healthy subjects (Figure 4.30). One of the dynamic parameters, $\gamma_{disc\text{-}min}$ 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 (Figure 4.30).

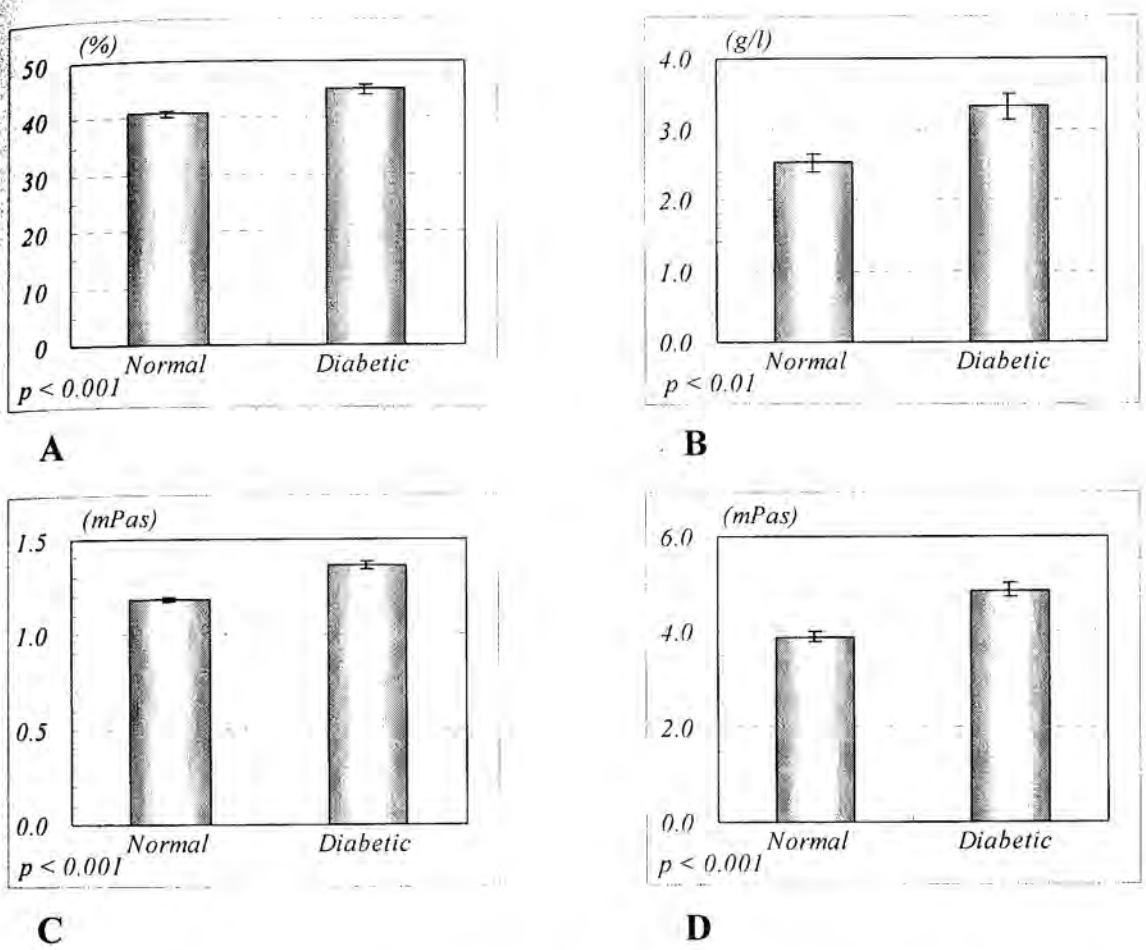

Figure 4.28 Hematocrit (A), plasma fibrinogen (B), plasma viscosity (C) and whole blood viscosity at 90 s^{-1} (D) of diabetic patients and healthy subjects.

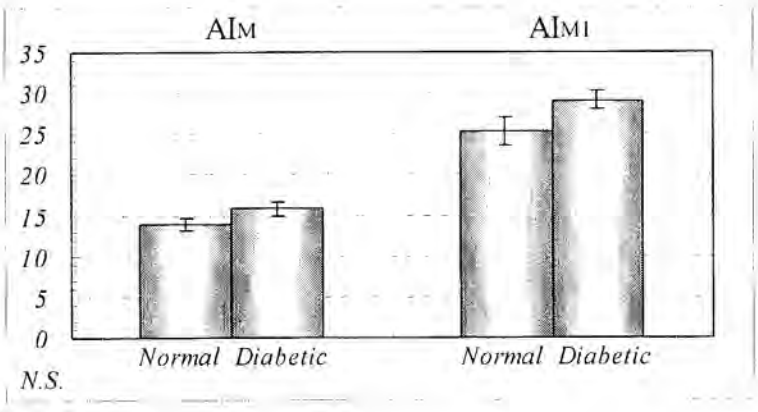

Figure 4.29 Aggregation indices measured by Myrenne aggregometer.

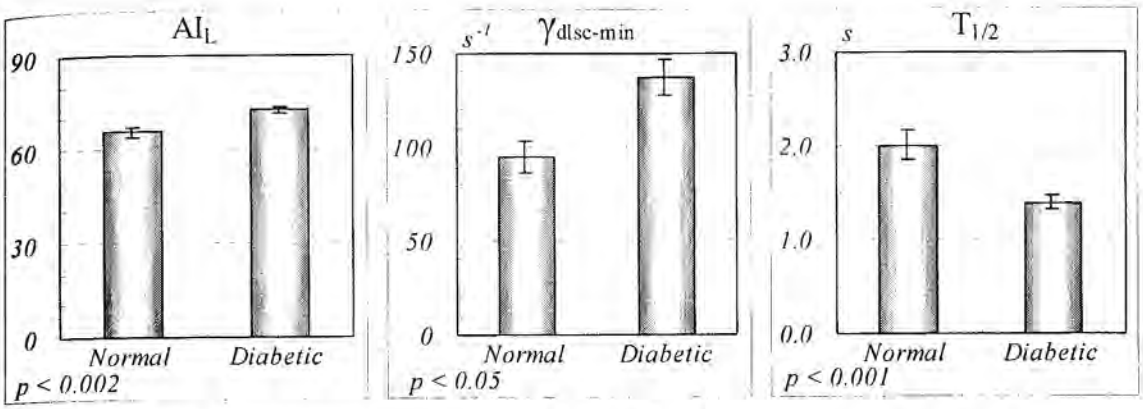

Figure 4.30 Aggregation parameters of LORCA; AI_L: aggregation index, $\gamma_{dlsc\text{-}min}$: minimum disaggregation shear rate, $T_{1/2}$: time to the half of the aggregation.

5. DISCUSSION

Hemorheological parameters are primary risk factors of vascular diseases such as cardiovascular, cerebrovascular and peripheral arterial diseases, hypertension and can also play a part in the development of vascular complications of diabetes mellitus.

Red blood cell aggregation is a reversible adhesion of red cells into three-dimensional rouleaux. The strength of aggregation was suggested to be directly proportional to the plasma concentration of fibrinogen and immunoglobulins, although at very high concentrations some saturation has been described. In certain parts of the microcirculation blood flow is rather slow, e.g. in post-capillary venules, thus slight red cell aggregation is normally observed in such regions. In conditions with raised fibrinogen or immunoglobulin level red blood cell aggregation can be increased that has significant hemorheological and microcirculatory effects, namely plasma and whole blood viscosity are increased, with the latter especially increased at low shear rates. Large three-dimensional aggregates can cause capillary plugging, elevate capillary pressure and flow resistance that can result in fluid extravasation, hemoconcentration, tissue oedema, capillary rupture, ischemia and hemorrhage. Therefore the accurate characterization of red blood cell aggregation is of clinical importance [11,62].

In our investigations closer correlation values between red blood cell aggregation and fibrinogen could be revealed by LORCA aggregometer comparing to Myrenne. It was also shown that elevated fibrinogen values exceeding a certain level (found to be 4.5 g/l in this study) did not result in further increase of red blood cell aggregation; moreover, a reduction in aggregation might occur, as excess amount of fibrinogen could hinder the aggregation process. Relatively low value of correlation coefficients can refer to that besides fibrinogen other factors (e.g. globulins, red blood cell deformability) also influence red cell aggregation.

Careful examination of plot diagram showed that at high fibrinogen concentrations AI_L values were in the highest range (although without a further elevation), whereas AI_{MI} values were in the middle of AI_{MI} range and AI_M values were at the lower part of its range. Although the principals of aggregation measurements are similar in both aggregometers, one of the main differences is the accurately adjusted temperature control of LORCA. The lower (room) temperature in Myrenne could decrease binding forces between cells, which can appear when too high concentration of molecules may hinder aggregation.

Slow blood flow can facilitate aggregation (represented by AI_{MI}), because it moves cells closer to each other, whereas complete stop of flow results in less and smaller aggregates

(AI_M); in LORCA the flow of the relatively high amount of blood between the vertical walls of cup and bob could not stop so abruptly, which can also promote aggregation forces. This can be the second reason why AI_M values at high fibrinogen concentration remains in the lower range: the balance between aggregation and disaggregation forces moves toward disaggregation.

In our studies aggregation measurements were carried out at the native hematocrit of the samples. Previous studies using Myrenne aggregometer implied the necessity of adjustment of sample hematocrit to a certain level. However, it should be noted that such adjustment could interfere with the aggregation process influencing the relatively weak forces between the cells. In the clinical practice it is also important that the test should be simple and short (considering especially the time limitation of the aggregation measurements). According to our results LORCA aggregometer system seems to yield clinically more relevant data. The reason of this can be the calculation of aggregation index in LORCA, which derives from the ratio of the area under the syllectogram curve and the area covered by the whole syllectogram. Therefore this index is independent from the absolute amplitude of the light intensity curve that is affected by the hemoglobin concentration of the sample (largely dependent on hematocrit) and oxygenization of the blood. Measurements in Myrenne had a coefficient of variation for repeated tests on the same sample of 5 %, while it was 3 % for LORCA [44].

Although there exist several instruments characterizing red blood cell aggregation, unfortunately, we do not have a "gold standard" method [4,21,27,44]. Myrenne aggregometer is the most widespread among those instruments, which characterize red blood cell aggregation photometrically. This could be due to that it was the first simply applicable device in this field. Its advantage is the very small amount of blood required for a measurement and the short measurement time (without sample preparation). Nevertheless, this device has its limits, e.g. the lack of temperature control, the limited amount of data on the aggregation process provided by the basic (not computerized) instrument, the calculation of an absolute area under the curve as an aggregation index without giving a ratio. LORCA is a more recently developed instrument, which has in many ways more sophisticated technique and several functions providing the possibility to determine red blood cell deformability also using the same device and other computer program. It is as simple to use as the Myrenne; although it needs more blood for one measurement, this amount does not exceed the size of a standard Vacutainer tube, which is generally used in clinical hemorheological measurements. At the same time, there has been even more limited amount of data on the clinical applications of LORCA comparing to Myrenne. Our study was the first that compared these

two instruments to each other. In the future investigations on red blood cell aggregation in many diseased states should be conducted in large populations using and comparing several devices on the market that can lead to the standardization of the measurement of this important process [6,22,29,57].

Coronary artery disease is both clinically and economically the most important part of vascular diseases, in which the role of impaired blood rheology is also emphasized. In our study all the measured hemorheological parameters were higher and the circulatory index was lower in patients with ischemic heart disease than in healthy controls, which is in concordance with the results of previous studies. Moreover, fibrinogen and plasma viscosity levels were more increased in the single vessel and multivessel disease group (G-2, G-3) comparing to IHD patients without significant coronary artery lesions (G-1), which shows the more deteriorated rheological state of those patients. The increased fibrinogen and plasma viscosity levels can result in higher red blood cell aggregability and all these changes can cause further reduction in coronary blood flow, which has already been affected by the stenotic lesions. Although the higher hematocrit value of the multivessel disease patients may support the oxygen transport, but on the other hand it can worsen the coronary circulation with the elevation of whole blood viscosity.

In spite of the statistically not different central hemodynamic parameters, circulatory index was found to be significantly lower in patients with IHD. Furthermore, it was significantly decreased in multivessel disease, which refers to the impaired circulation even in resting subjects. In our previous studies circulatory index was significantly reduced in ischemic heart disease both at rest and during exercise, and it was also proved to be a more sensitive parameter for the characterization of the state of circulation than whole blood viscosity or cardiac index alone [66].

There were smaller number of patients in G-1 and G-2. This lower rate of patients with less severely affected coronaries is unfortunately characteristic in our country, where most of the patients undergoing invasive procedures have poor coronary vessel state. Therefore the subjects in G-1 could not be classified into subgroups. Studies should be performed in order to examine the hemorheological parameters in these patients with minor (not significant) lesions on coronary arteries, with coronary spasm or coronary X syndrome, because their coronary flow can be reduced and rheological alterations can cause even further reduction.

In the Edinburgh Artery Study a relationship between hemorheological factors and carotid intima-media thickness could be revealed in men. Carotid intima-media thickness is a

marker of the early, subclinical stage of atherosclerosis. The association was independent of other important risk factors: total cholesterol, blood pressure and smoking. Of the hemorheological variables, fibrinogen may promote atherosclerosis through an increase in platelet aggregation, fibrin formation, and blood viscosity and decreased fibrinolysis. Elevated blood viscosity may in turn promote platelet adhesion to the endothelium, increase protein filtration into the arterial wall, and alter local shear forces at sites of atherogenesis. The Scottish group explained the greater susceptibility of men to viscosity with sex differences in vascular geometry and wall shear forces [37]. Habon et al. showed that the evaluation of hemorheological variables along with the other diagnostic procedures can help with clinical decision-making in persons with suspected ischemic heart disease [25]. Neumann et al. described a relation between impaired blood fluidity and chronic coronary artery disease, which was found to be independent of the extent of coronary atherosclerosis. They found that plasma viscosity and red blood cell aggregation were higher in unstable angina and the marked elevation of these parameters could identify a subgroup of patients with unstable angina at a high risk of acute myocardial infarction [48,49]. Corresponding with our results, Junker et al. showed plasma viscosity to be related to the severity of coronary artery disease. Nevertheless, they examined men only; the absolute values of plasma viscosity in their study were lower than in ours and other studies because of the use of citrate diluted plasma in the measurements. They suggested plasma viscosity as a linking mechanism between other cardiovascular risk factors and coronary heart disease [30].

In summary, our findings indicate not only that hemorheological parameters are important risk factors of ischemic heart disease, but also that they can change along with the severity of the coronary artery disease and may play a pathophysiological role in the deterioration of this disease.

In the PTCA study hematocrit, plasma and whole blood viscosity decreased significantly thirty minutes after the procedure that could be caused by hemodilution. This could be due to the several blood samplings, some blood loss caused by the catheterization technique, infusions and contrast agents, respectively. Since these changes were transient, they were not supposed to have significant advantageous effect on the coronary circulation. Corrected whole blood viscosity, a simple mathematical correction of whole blood viscosity to a standard hematocrit that allows the comparison of viscosities with different hematocrit values, did not reduce after angioplasty, which supports the idea of hemodilution [33].

Hematocrit showed a gradual increase during the days and months following the procedure, which was significant after six months. The elevation of plasma viscosity was parallel with the changes of fibrinogen level until the second day, but then seemed to remain higher than baseline until the sixth month. Whole blood viscosity showed a similar trend to that of hematocrit, although its elevation became significant by the end of the first month. The background of this deterioration in hemorheological parameters after six months is still obscure; nevertheless, these changes are similar to the results found in previous studies of Tóth et al. in patients with myocardial infarction [64,65]. The elevation of corrected blood viscosity may refer to an impairment of the erythrocytes, which (beside the more frequently examined platelets or leukocytes) may also be affected by the procedure.

Fibrinogen has been proved to be an independent risk factor of cardiovascular diseases [9,20,34,47]. In a recent study fibrinogen and von Willebrand factor were proposed to be risk factors of restenosis after PTCA [46]. In our study plasma fibrinogen level elevated markedly on the first two days after PTCA, then decreased to the baseline. These changes can be explained by an acute phase reaction caused by the procedure, as fibrinogen is a well-known marker of this process. Acute phase reaction occurs after organic lesion, as a part of systemic inflammatory response. Several mechanisms can account for the inflammatory response induced by angioplasty: plaque rupture, hemorrhage, arterial wall damage, release of inflammatory and chemoattractant factors from activated platelets and leukocytes, repeated ischemia and reperfusion due to several balloon inflations and deflations. In a recent study another acute phase marker, C-reactive protein was found to be increased two days after coronary angioplasty [1]. Plasma fibrinogen concentration is known to elevate following unstable angina and acute myocardial infarction, and higher values are associated with poorer outcome. The elevation in fibrinogen after PTCA might increase the risk of acute ischemic coronary events, since fibrinogen plays a central role in platelet and erythrocyte aggregation and is one of the main determinants of plasma and whole blood viscosity. More interestingly, plasma fibrinogen was elevated at the six-month sampling in correspondence with the other rheological parameters. The reason of this change is still not known (there was not any significant change in the treatment and lifestyle of the patients), so it needs further clarification whether this could elevate the risk of coronary events.

Our study involved a relatively low number of patients; therefore relationship between hemorheological parameters and restenosis could not be evaluated. Red blood cell properties were characterized indirectly by hematocrit, whole blood and corrected blood viscosity;

therefore the measurement of red blood cell aggregability and deformability could reveal interesting new details.

In summary, we found significant changes in hemorheological parameters after percutaneous transluminal coronary angioplasty, which can deteriorate the coronary circulation and affect the final outcome of this intervention.

The results of the PTCA study indicate also that, as a consequence of the procedure, there is cellular activation involving platelets and leukocytes. In recent studies increased expression of neutrophil, monocyte and platelet adhesion molecules was found [16,36,60]. Signs of enhanced lipid peroxidation and reduction of antioxidant activity may confirm the presence of oxygen derived free radical mediated reactions after coronary angioplasty, which may evoke further cellular and tissue damage. Previous studies could reveal significant changes in plasma lipid peroxide concentrations in patients with coronary artery disease or myocardial infarction [55,73], whereas no change in this parameter could be found in a PTCA study [51]. Although the non-specific TBARS determination was applied as a marker of lipid peroxidation, in a study analyzing both the HPLC and the TBARS techniques, good correlation could be found between their results [55].

Activation of white blood cells at the site of the procedure as well as in the vessels downstream implies their adhesion to the vascular wall, plugging of capillaries, and generation of free radicals and chemoattractant factors, which affect red blood cells damaging their membrane and reducing their deformability, attract and activate platelets [16,41]. All these changes associating with the worsening of the hemorheological factors can deteriorate the circulation of the myocardium [58]. It should be noted that platelet activation is shear-dependent that also emphasizes the role of the hemorheological conditions. In a dog model the perfusion of blood containing red cells with reduced deformability into the coronary circulation decreased coronary flow markedly and impaired cardiac function. The changes were attributed partially to capillary plugging and the role of platelet activation also was proposed, because the harmful effects could markedly be reduced with aspirin treatment [23]. (Bilto recently reported a direct advantageous effect of aspirin on erythrocytes [8].)

Spontaneous platelet aggregation was shown to be a strong determinant of survival in patients with acute myocardial infarction [35,70]. In our study spontaneous platelet aggregation was increased significantly in the samples from the coronary sinus before the procedure, which may refer to the activation of platelets in the diseased coronaries. Moreover, there was a further elevation in this parameter thirty minutes after PTCA, which returned to

the baseline two days later. All these findings confirm that PTCA can result in platelet activation and also imply that the conventional antiplatelet combination (aspirin + ticlopidine) may be insufficient to achieve a marked reduction in platelet aggregation and thrombus formation in cases when enhanced platelet activation occurs. These findings also indicate the necessity of new antiplatelet agents (e.g. glycoprotein IIb/IIIa receptor blockers, etc.) in the routine clinical practice.

In patients with diabetes mellitus and diabetic retinopathy the hemorheological variables were markedly worse than in control persons. These finding are similar to those observed by Vékási et al. in a group of patients with hypertensive retinopathy [71]. Besides the macrorheological parameters (hematocrit, plasma and whole blood viscosity), red blood cell aggregation of diabetics measured in LORCA was significantly increased compared to that of healthy subjects that could be due to the higher plasma fibrinogen concentration. Our results indicate that not only the extension of aggregate formation is larger (creating more and bigger aggregates), but also the aggregating process is faster (represented by $T_{1/2}$) and higher shear is necessary to disrupt the aggregates (represented by $\gamma_{dlsc\text{-}min}$). The increased red blood cell aggregation means that blood flow could be most affected in areas of low shear stress such as capillaries and post-capillary venules that can cause plugging and stasis in these vessels. This investigation shows also that LORCA aggregometer is a valuable instrument in the characterization of the hemorheological conditions of a disease in a clinical study. AI_M and AI_{M1} values of Myrenne showed an increasing tendency, although the results were not statistically different. This could be caused by the relatively higher deviation of the results, because of the low number of subjects especially in the control group and the higher dependency of the Myrenne on measurement circumstances (written more detailed previously). This Myrenne aggregometer was not a computerized version; therefore dynamic parameters ($T_{1/2}$ and γ) could not be determined and compared to the results of LORCA.

It should be noted that mean fasting serum glucose concentration was markedly higher than normal in these patients, in spite of that their disease had been known for several years and they were on antidiabetic treatment. Previous studies gave conflicting results regarding the relationship of metabolic control and hemorheological parameters. Because of the high glucose level, increased diuresis can cause hemoconcentration resulting in increased hematocrit and whole blood viscosity values. On the other hand, glucose concentration can vary more rapidly during a day that is not necessarily followed by rapid changes in

hemorheological conditions, but their deteriorated values could reflect the metabolic disturbances of a longer period or the diseased state in general. Higher rate of glycosilation can decrease the deformability of red blood cells. The results of patients with non-proliferative and proliferative retinopathy were similar to each other, which may imply that pathological hemorheological values could be involved in the development of the complication at the early stage.

The elevated hemorheological parameters associated with the other changes observed in diabetes mellitus such as increased activity of neutrophil granulocytes, endothelial cell injury, elevated expression of cell adhesion molecules, and injured neurogenic regulation of microvessels can cause angiopathies and severely damage the circulation of the organs. Therefore the amelioration of the hemorheological factors could be helpful in the prevention of complications in this disease.

6. 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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