

## 11. DISTURBANCES OF HAEMOSTASIS IN DIABETES MELLITUS CONTRIBUTING TO THE DEVELOPMENT OF ATHEROSCLEROSIS

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### 1.1. Introduction

Diabetes mellitus is a chronic condition characterized by the presence of fasting hyperglycaemia and the development of widespread premature atherosclerosis. Patients with diabetes have increased morbidity and mortality due to cardiovascular diseases, especially for coronary artery disease. Vascular complications in diabetes may be classified as microvascular, affecting the retina, kidney and nerves and macrovascular, predominantly affecting coronary, cerebrovascular and peripheral arterial circulation.

Typically, diabetes mellitus type 1 (insulin-dependent) occurs in young, slim individuals in whom pancreatic function is absent, whereas diabetes mellitus type 2 (non-insulin-dependent) occurs in the middle-aged, obese population. In diabetes mellitus type 2, insulin secretion, though abnormal, is preserved and insulin resistance is a common feature. Insulin resistance influences several haemostatic factors, the effect being greatest in respect to the fibrinolytic system. Chronic hyperglycaemia results in hyperglycosylation of multiple proteins and is the hallmark of diabetes. Hyperglycosylated proteins have altered function resulting in a spectrum of effects.

The pathogenesis of the atherosclerosis in diabetes mellitus is not entirely clear and conventional risk factors such as smoking, obesity, blood pressure and serum lipids fail to explain fully this excess risk. Important features in the pathogenesis of atherosclerosis appear to include vascular endothelial injury, platelet adhesion and activation, fibrin deposition, cellular proliferation, and low-density lipoprotein cholesterol accumulation. Fibrin deposition is an invariable feature in atherosclerotic lesions. Therefore, disturbances of haemostasis leading to accelerated fibrin formation (hypercoagulability) and delayed fibrin removal (impaired fibrinolysis) may contribute to the development of atherosclerosis. Hyperactive platelets, hypercoagulability and impaired fibrinolysis also promote thrombosis formation at the site of ruptured atherosclerotic lesion and lead to final occlusion event in the progression of atherosclerosis.

### 1.2. Hyperactivity of platelets

Although platelet counts are normal in patients with diabetes mellitus, multiple studies offer evidence of enhanced activation or increased platelet activity. Additionally, an increase in plasma levels of von Willebrand factor (vWF), which is important for the adhesion of platelets to subendothelial structures, has been reported in diabetic patients. Hyperactive platelets may form microaggregates leading to capillary microembolization. In patients with diabetes the resulting relative tissue hypoxia may in the long-term precede clinically detectable microangiopathy. It has been speculated that microembolization of the vasa vasorum

of the large vessels by hyperactive platelets may also be the initial event in the development of atherosclerosis. Secretion of mitogenic, oxidative or vasoconstrictive substances by platelets activated in response to endothelial injury amplifies and accelerates the progression of atherosclerosis. Acute thrombotic events in the arterial circulation are also triggered by platelets.

Platelets of patients with diabetes mellitus type 2 are hypersensitive to agonists, which cause platelet aggregation, such as ADP and arachidonic acid. In diabetes mellitus type 1, increased response to ADP was observed. A number of mechanisms could contribute to this hypersensitivity. Increased presence of glycoprotein receptors GPIb and GPIIb/IIIa for agonists and adhesive proteins on the platelet surface is one of them. Increased fibrinogen binding was also observed in diabetic patients but platelets did not show increased receptor numbers. There is some evidence for increased platelet activity in vivo in diabetes, but it is unclear whether this reflects platelet hypersensitivity or increased platelet turnover on already diseased vessels.

Activated platelets release multiple chemical substances and proteins from their dense and alpha granules. Levels of some of these products serve as markers of in vivo platelet activation. Various studies have found high levels of thromboxane A<sub>2</sub>, b-thromboglobulin, platelet factor 4 and fibronectin in patients with diabetes.

### **1.3. Increased coagulation factors and hypercoagulability**

In diabetes mellitus disturbances of haemostasis leading to hypercoagulability have been observed in numerous studies. Besides altered screening tests, alterations of several coagulation factors and inhibitors have been occasionally described. The problem encountered when studying the association between hypercoagulability and atherosclerosis is the great number of laboratory tests proposed to detect hypercoagulability and the wide variability of such tests in a given subject. Results of cohort studies have shown that among different coagulation factors analyzed, increased concentration of fibrinogen, factor VII and vWF have predictive value for coronary atherosclerosis and can be considered as risk factors for cardiovascular events. Increase in these factors could participate in the pathogenesis of atherosclerosis, predominantly of coronary arteries.

Fibrinogen is a parameter that has been studied most extensively in epidemiological studies. A relationship has been established between plasma concentration of fibrinogen, the quantity of fibrinogen and fibrin present in the vessel wall and the severity of atherosclerosis. These associations are more pronounced in diabetic patients. Plasma fibrinogen concentration is influenced by environmental factors - mainly by smoking and age. Fibrinogen, which is an acute phase protein, is increased in winter (possibly due to infections), in obese subjects, in pregnant women, in women during menopause and in women using oral contraception. High fibrinogen concentration is observed also in diabetic patients, especially in those with albuminuria. Relationship between fibrinogen and insulin resistance is controversial. Free fatty acids have been suggested to explain the fibrinogen - insulin resistance relationship, because a simultaneous increase in free fatty acids and fibrinogen is seen in variety of clinical and experimental condition. This relationship might also result from an inflammatory

reaction accompanying atherosclerosis.

Factor VII is a vitamin K dependent protein synthesized in the liver. It is the key enzyme in the initiation of blood coagulation. The Northwick Park Heart Study and the PROCAM study have shown that there is a positive correlation between increased factor VII and cardiovascular mortality. Plasma concentration of factor VII is closely related to several environmental factors, mainly triglycerides and cholesterol levels. These associations are highly dependent on dietary intake. An increase in factor VII has been described in diabetes mellitus and is more pronounced in those with microalbuminuria. Only limited data are available concerning the contributory role of insulin resistance to elevated factor VII. The relationship between factor VII and insulin and proinsulin have been described as very weak or present only in women. Factor VII which is influenced by the efficiency of the metabolism of triglyceride-rich lipoproteins could in this way be modified in insulin resistance.

Increased plasma concentration of vWF has been shown to be predictive of re-infarction and mortality in survivors of myocardial infarction, of cardiac events in healthy people and in patients with angina pectoris. The European Concerted Action on Thrombosis study showed that vWF predictability was not affected by the adjustment with other classical coronary risk factors such as body mass index, lipid disorders or smoking. As vWF levels are dependent on the acute phase reaction like fibrinogen, and vWF correlates positively with fibrinogen or C-reactive protein levels, it has to be evaluated if vWF is a risk factor irrespective of fibrinogen level. In type 2 diabetic patients vWF levels are higher in microalbuminuric patients. vWF is very poorly or not at all related to insulin resistance.

Hypercoagulability can be judged also from increased levels of markers of coagulation system activation, which reflect enhanced thrombin generation. Prothrombin fragment 1+2 released when thrombin is formed from prothrombin is increased in diabetes. Once activated, thrombin is rapidly inactivated by antithrombin, forming thrombin-antithrombin complexes, which subsequently circulate and are removed by the liver. Multiple studies have documented elevated thrombin-antithrombin complexes in diabetes. Fibrinopeptide A is released when fibrinogen is converted to fibrin by thrombin. Thus, fibrinopeptide A levels are increased during coagulation. Measurement of fibrinopeptide A in diabetes has yielded a variety of results, from elevated to normal.

#### **1.4. Disturbances of fibrinolysis**

The fibrinolytic system is natural defence against thrombosis. A balance exists between plasminogen activators and inhibitors, and impairment of this balance can be caused either by diminished release of tissue plasminogen activator (t-PA) or increased levels of plasminogen activator inhibitor 1 (PAI-1). PAI-1 is a serine protease inhibitor and evidence suggests that it is the major regulator of the fibrinolytic system. It binds and rapidly inhibits both single- and two-chain t-PA and urokinase. t-PA and PAI-1 rapidly form an inactive irreversible complex.

Abnormalities of the fibrinolytic system have been described in both diabetes mellitus type 1 and type 2. Impaired fibrinolysis, as described in diabetes type 2, is commonly accompanied by an increased plasma levels of PAI-1 and by increased concentration of t-PA antigen,

which reflects predominantly t-PA/PAI-1 complexes. In diabetes mellitus type 1 results are mixed, and diminished, normal and enhanced fibrinolysis have all been reported.

In subjects with diabetes mellitus type 2 a variety of risk factors are independently associated with impaired fibrinolysis: obesity, hypertension, dyslipidaemia, glucose intolerance, hyperinsulinaemia and insulin resistance. These factors often tend to converge and numerous studies have attempted to dissect out the independent contribution of the above risk factors in determining fibrinolytic activity in diabetes, but this task has been hampered by the complex relationship between them. In non-diabetic subjects, insulin resistance is paralleled by increased insulin and both correlate with triglyceride levels. Thus any one or more of these variables may explain interrelationship with PAI-1. By contrast in diabetes type 2, insulin resistance, insulin concentration and triglyceride levels are less tightly interdependent in explaining increased PAI-1.

Impaired fibrinolysis not only predisposes to thrombotic events but also plays a role in the formation and progression of atherosclerotic lesions. Increased synthesis of PAI-1 has been demonstrated in atherosclerotic lesions. This may lead to fibrin deposition during lesion rupture, contributing to the progression of the lesion. PAI-1 within the lesion inhibits plasmin formation, which plays an important role in cleaving extracellular matrix proteins, directly or via activation of metalloproteinases. This may lead to stabilization and further growth of atherosclerotic lesion.

Changes in the fibrinolytic system also play an important role in microangiopathy. Urokinase and plasmin are activators of latent metalloproteinases, such as collagenases, that are responsible for proteolysis of extracellular matrix proteins. Increased PAI-1 may lead to basement membrane thickening observed in microangiopathy.

Hyperinsulinaemia has been associated with cardiovascular disease in non-diabetic subjects. In those with diabetes mellitus type 2 the extent of hyperinsulinemia parallels plasma PAI-1 activity, and insulin has been implicated as a major physiological regulator of PAI-1. Despite population correlations of insulin and PAI-1, and the effect of insulin on PAI-1 production in vitro, a direct effect of insulin on PAI-1 levels in vivo in humans has not been shown, either with intravenous infusion of insulin or by an oral glucose load with the aim of producing portal hyperinsulinemia. Thus, in humans there is little evidence that interventions resulting in increased concentration of insulin in vivo increase PAI-1. On the other hand reducing insulin levels and insulin resistance by exercise, weight loss and the drug metformin has been shown to reduce PAI-1. In patients with diabetes mellitus type 2 approximately 30 % of fasting immunoreactive insulin concentration consists of proinsulin-like molecules. The elevated levels of PAI-1 in these subjects may, therefore, be a consequence of precursor insulin rather than insulin itself.

In non-diabetic subjects, increased insulin is associated with insulin resistance. In patients with diabetes mellitus type 2 this association is less close. In a study of nine patients PAI-1 levels have shown an inverse correlation with insulin sensitivity. However, the relationship of hyperinsulinemia and insulin resistance with elevated PAI-1 is yet to be unraveled.

Hyperglycaemia is an additional risk factor for impaired fibrinolysis. Glucose can directly in-

crease PAI-1 production in human endothelial cells. In patients with diabetes mellitus type 2 a significant correlation between glucose concentration and PAI-1 and has been observed.

It has been proposed that insulin resistance or hyperinsulinemia could influence the synthesis of PAI-1 via effects on lipid metabolism. In patients with diabetes mellitus, dyslipidaemia, in particular high triglyceride and low high-density lipoprotein level, is common. Studies in vitro have demonstrated the effect of various lipoproteins on PAI-1 synthesis. Very-low-density lipoproteins from hypertriglyceridaemic patients increase endothelial cell production of PAI-1 to a greater degree than that from normo-triglyceridaemic subjects. Oxidized low-density lipoproteins also stimulate endothelial cell PAI-1 synthesis as does lipoprotein(a). Lipoprotein(a), low-density lipoprotein, and high-density lipoproteins also suppress t-PA secretion from human endothelial cells in dose dependent manner.

### **1.5. Conclusion**

There is significant laboratory evidence of chronic platelet activation, enhanced coagulation and impaired fibrinolysis in patients with diabetes mellitus. These disturbances of haemostasis favour development of atherosclerosis and thrombosis in particularly of coronary arteries. In the future with better understanding of molecular mechanisms that regulate haemostasis, it may be possible to identify high risk diabetic patients, candidates for early interventions before the development of vascular disease.

#### **Recommended literature:**

1. Carr ME. Diabetes mellitus. A hypercoagulable state. *J Diabet Compl* 2001; 15: 44-54.
2. Juhan-Vague I, Alessi MC, Vague P. Thrombogenic and fibrinolytic factors and cardiovascular risk in non-insulin-dependent diabetes mellitus. *Ann Med* 1996; 28: 371-380.
3. Mansfield MW, Grant PJ. Fibrinolysis and diabetes mellitus. In: Glas-Greenwald P (ed). *Fibrinolysis in disease. Molecular and hemovascular aspects of fibrinolysis*. CRC Press, Boca Raton-New York-London-Tokyo 1996, pp 172-83.
4. Panahloo A, Yudkin S. Diminished fibrinolysis in diabetes mellitus and its implication for diabetic vascular disease. *J Cardiovascular Risk* 1997; 4: 91-9.
5. Tschoepe D, Roesen P, Schwippert B, Gries FA. Platelets in diabetes: The role in the hemostatic regulation in atherosclerosis. *Sem Thromb Haemost*