

Review / Revisão

Hemostatic changes in patients with type 2 diabetes mellitus

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Diabetes has acquired an epidemic character due to the large increase in the number of individuals affected over recent decades. Diabetes-related mortality is associated with thrombotic events, especially cardiovascular. In general, patients with diabetes present symptoms of hypercoagulability and hypofibrinolysis. However, the mechanisms that trigger hemostatic abnormalities in diabetic patients are not clear. The aim of this paper was to address the most frequent changes of the hemostatic system in diabetic patients described in the literature. Diabetics have abnormalities of the endothelium, platelets, clotting factors, natural anticoagulants and the fibrinolytic system; all these changes are directly and/or indirectly caused by hyperglycemia. Thus, analytes such as von Willebrand factor, factor VIII, fibrinogen and D-dimer are markers that should be interpreted differently in diabetic patients. Laboratory evidence of hemostatic abnormalities in diabetic patients supports clinical observations that diabetes is a state of hypercoagulability and hypofibrinolysis. Strategies for clinical intervention and medications are not well established considering the results of the hemostatic markers.

Keywords: Diabetes mellitus, Type 2; Hemostasis; Thrombophilia; Fibrinolysis

Introduction

Due to the increasing number of diabetics, this disease has acquired a character of "epidemic" in recent decades. In 2000, the number of diabetics worldwide was approximately 151 million; estimates are that in 2010 this will reach 221 million and by 2025, 324 million.⁽¹⁾ It is believed that changes in human behavior, environment and lifestyle are favoring an increase in the number of obese and diabetic individuals.⁽²⁾

Two types of diabetes mellitus are the most prevalent: type-1 diabetes is characterized by autoimmune destruction of pancreatic beta cells resulting in an absolute deficiency in insulin; and type 2 diabetes (T2DM), which corresponds to

approximately 90% of cases of diabetes worldwide, is characterized by insulin resistance and/or reduced production of insulin.⁽²⁾

The diabetes epidemic is due, in particular, to T2DM. Paradoxically, part of the problem is related to improvements of public health programs during the 20th century and increased life expectancy. Diseases that are not of mandatory notification, including diabetes and cardiovascular disease, are currently the greatest public health problem responsible for the increased morbidity and mortality seen in the 21st century.⁽²⁾

The costs involved in treating diabetes and associated complications are high, thus, heavy investments have been made in primary prevention (to reduce the

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incidence of DM) and secondary prevention (to reduce the immediate and long-term complications of diabetic patients). The treatment of hypertension and dyslipidemia and the control of hyperglycemia are basic measures to prevent the development of diabetes-related complications.⁽²⁾

The major complications resulting from T2DM are related to the microvascular and macrovascular systems.^(3,4) The most common microvascular complications are nephropathy, retinopathy and neuropathy and the most important macrovascular complications include coronary artery disease, strokes and peripheral arterial disease.⁽⁵⁻⁷⁾

About 80% of diabetics die from thrombotic events with 75% to 80% of these deaths resulting from cardiovascular events.^(8,9)

Diabetic patients without any prior infarction event are at risk of cardiovascular disease similar to non-diabetic subjects with a history of infarction. Treatment of high-risk patients reduces the chance of cardiovascular disease bringing the risk in line with individuals without diabetes.⁽¹⁰⁾ In general, diabetic patients present symptoms of hypercoagulability and hypofibrinolysis. However, correlations between the vascular complications related to diabetes and the degree of abnormality of the haemostatic system have not been clearly established.⁽³⁾ The aim of this article was to address the most common changes to the hemostatic system of T2DM individuals as described in the literature.

Endothelial changes in diabetes

The endothelium consists of a single layer of cells lining the inside surface of blood vessels, hence establishing a barrier between the blood and the vessel.⁽¹¹⁾ The endothelium contributes to maintaining the blood flow by preventing platelet aggregation, its anticoagulant properties and by stimulating the fibrinolytic system.⁽¹²⁾ Changes in the endothelium can activate inflammatory processes, which together with other factors such as hypertension and dyslipidemia, cause atherosclerotic plaques. These plaques may remain asymptomatic for years and not cause any clinical changes in diabetic patients.⁽¹³⁾

Hyperglycemia directly contributes to endothelial injury through irreversible glycation of collagen and other subendothelial structural proteins of the vessel, forming advanced glycation end products (AGEs).⁽¹⁴⁾ AGEs accumulate in the subendothelium over time influenced by increases in blood sugar levels and are directly related to atherosclerosis and renal failure.^(15,16) AGEs cause changes in structure and biophysical properties of the basement membrane causing changes in permeability and vasodilation of blood vessels.⁽¹⁷⁾

One method to assess vascular integrity is by the measurement of plasma thrombomodulin (TM) since this is essentially a membrane protein.⁽¹⁸⁾ Thus, increased plasma

TM levels may provide laboratory evidence of endothelial injury and, indirectly, a reduction in the effectiveness of the protein C anticoagulant pathway.⁽¹⁹⁾ Particularly in T2DM patients, increased levels of TM appear to be associated with a diffuse vascular injury that is not tissue-specific.⁽²⁰⁾

Another marker of endothelial injury is the von Willebrand factor (vWF).⁽²¹⁾ In some conditions such as hypertension and hyperinsulinemia, vWF levels are elevated; the vWF levels drop in coronary artery disease patients when they start treatment to reduce hypercholesterolemia.⁽²²⁾

The vWF is a multimeric glycoprotein that synthesized by megakaryocytes and endothelial cells and is present in the subendothelium, plasma, platelets and endothelium. Secreted in a high molecular weight form, vWF is cleaved by ADAMTS13 into smaller multimers.⁽²³⁾ Within the endothelial cell, the multimers are stored in Weibel-Palade bodies,⁽¹¹⁾ and so when endothelial injury occurs, a notable increase in vWF levels occurs and a possible hypercoagulable state may be set off.⁽²⁴⁾

Epidemiological studies show that high vWF levels predict the evolution or progression of cardiovascular disease; intervention studies have shown that treatment of hypercholesterolemia, hypertension, diabetes and hyperhomocysteinemia reduce vWF levels as does smoking cessation.⁽²²⁾ An increase in vWF has been observed preceding T2DM⁽²⁵⁾ and there is a positive association between increased vWF and progression to microvascular⁽²⁰⁾ and macrovascular⁽²²⁾ dysfunction in diabetes.

Chronic hyperglycemia or the various associated metabolic abnormalities, such as hypertension, dyslipidemia and hyperinsulinemia may cause endothelial injury resulting in microvascular lesions characteristic of diabetes such as nephropathy. Additionally, there is a hypothesis that microalbuminuria in diabetic patients does not only indicate renal injury, but also widespread vascular damage.⁽²⁰⁾

All these factors together explain the positive association between microalbuminuria, increases in TM and vWF, and cardiovascular events because, in diabetes, these factors are associated with microvascular and macrovascular lesions.⁽²⁴⁻²⁶⁾

Other functions directly associated to endothelial dysfunction are also impaired. The enzyme, lipoprotein lipase, needs to bind to endothelial glycosaminoglycan molecules to function, and changes in enzyme binding to the endothelium may contribute to diabetes-related dyslipidemia.⁽²⁴⁾

Platelet changes in diabetes

Platelets are discoid-shaped (when not activated) cytoplasmic fragments of megakaryocytes that have a mean

diameter of 2 to 3 μm and an average life of six to ten days.^(27,28)

A platelet plug forms after endothelial injury. The dynamics of platelet plug formation involves several steps including adhesion, change in shape, aggregation and platelet granule secretion. After platelet adhesion to the subendothelium mediated by glycoprotein Ib (GPIb) with vWF on subendothelial collagen, there is a change in platelet shape and previously internalized, negatively-charged phospholipids and receptors are exposed. The release of adenosine diphosphate (ADP) from dense granules together with the mobilization of calcium results in conformational changes in the platelet that exposes glycoprotein IIb/IIIa (GPIIb/IIIa), allowing platelet-platelet interaction mediated by fibrinogen molecules. This process initiates platelet aggregation. The secretion of granules signals the recruitment of other platelets to the vessel wall resulting in platelet plug formation.^(28,29) The purpose of platelet plug formation is to isolate the injury site.^(27,28)

The number of circulating platelets in diabetic patients is normal, i.e. there is no quantitative change compared to the non-diabetic population.⁽⁸⁾

Although hyperglycemia, dyslipidemia and hypertension may independently cause vascular damage, endothelial dysfunction may be intrinsic to T2DM. This condition can lead to an activated state characterized, in part, by platelet adhesion and increased aggregation.⁽³⁰⁾

Moreover, the osmotic effect of glucose consists in a mechanism by which hyperglycemia increases the propensity of platelets to aggregate and degranulate.⁽³¹⁾

It is not clear from the literature whether platelets interact more intensely in the affected vessels of diabetic patients, however, this fact may contribute to an increased propensity to arterial thrombotic events. Studies evaluating *in vitro* platelet function of samples from diabetic subjects found increased reactivity, increases in the numbers of GPIb and GPIIb/IIIa molecules and a reduction in membrane fluidity correlated with glycation of platelet membrane proteins.^(32,33)

In vivo studies have shown evidence of increased platelet activation in patients with metabolic syndrome and T2DM due to increased levels of beta-thromboglobulin and platelet factor 4 in the plasma; these markers are only stored in platelet granules.⁽¹³⁾

An increase of P-selectin (CD62P) on the platelet surface of diabetic individuals has also been described.⁽³⁰⁾ This is a platelet activation marker directly related to the formation of thrombi.

The prophylactic use of antiplatelet agents has been discussed and often justified for some patients, given that diabetes rarely occurs as an isolated risk factor for thrombosis. The most important risk factors associated with diabetes include: obesity, smoking, hypertension, dyslipidemia, advanced age, recurrent infections,

immobilization, malignancies, menopause and oral contraceptive use.⁽¹⁾

Changes in coagulation factors in diabetes

Cohesion in platelet plugs is insufficient to stop bleeding from large vessels or resist intravascular pressure in the arterial system. Under these circumstances, the formation of a fibrin clot, supported by the platelet plug, is necessary.⁽³⁴⁾

Under normal conditions, proteins and cellular components involved in blood clotting are present in blood but in inactive forms. When activated, there is a cascade of reactions that, through the generation of proteases, culminates in the conversion of fibrinogen into fibrin.⁽³⁵⁾

Metabolic syndrome subjects and T2DM patients have increased Factor VII (FVII) levels. This increase in FVII is related to the dyslipidemia present in both conditions. There is a positive correlation between FVII and triglycerides, with one hypothesis being that part of FVII circulates in plasma bound to very low-density lipoprotein particles rich in triglycerides, thereby prolonging the plasma half-life of FVII.^(13,21,36)

The use of statins (inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase), in addition to exerting direct effects on lipids, also reduces plasma levels of FVII thus regulating coagulation.⁽³⁷⁾

The factor VIII/vWF complex is also increased in individuals with insulin resistance and T2DM. This increase may be related to the presence of endothelial dysfunction and/or an inflammatory process, since vWF is an acute phase protein that is stored in the form of multimers in endothelial cells.^(13,14,21)

About 95% of factor VIII (FVIII) circulates in plasma bound to vWF and 5% circulates free.⁽³⁸⁾ Plasma levels of vWF are mainly related to endothelial cell secretion and due to inflammation in response to tumor necrosis factor (TNF). FVIII also increases in inflammatory states however it is believed that this is probably due to vWF increases, its carrier protein, since the FVIII synthesis is not altered by inflammation.⁽²¹⁾ Mathematical models estimate that the FVIII half life increases from 37 minutes to 24.5 hours when it is bound to vWF.⁽²¹⁾ The mean physiological half life of intravenously infused FVIII is approximately 12 hours; this may be influenced by vWF levels, the blood group, age, FVIII structural changes and alfa-2-macroglobulin receptor (CD91) gene polymorphisms.^(38,39)

An increase in FVIII plasma levels is considered an independent risk factor for thromboembolism.⁽⁴⁰⁾

Fibrinogen, another acute phase protein, is increased in diabetic patients.^(14,21,24,41) An increase in fibrinogen plasma levels is also considered an independent risk factor for cardiovascular disease.⁽³⁶⁾

It is well known that insulin therapy does not alter

fibrinogen levels, however, metformin therapy results in significant reductions in fibrinogen in T2DM patients.⁽¹³⁾

Another direct effect of hyperglycemia on the hemostatic system is observed in the fibrinogen molecule. The glycation of fibrinogen results in the formation of a denser fibrin clot with finer fibers that is resistant to fibrinolysis. The glycated fibrin binds less to both tissue-type plasminogen activator (t-PA) and plasminogen and generates less plasmin but binds more to alpha2-antiplasmin.^(8,13)

Hypercoagulability markers, such as prothrombin fragment 1 + 2 (F1 + 2), thrombin-antithrombin complex (TAT) and fibrinopeptide A (FPA) are also elevated in diabetic patients with comorbidities that include hypertension, cerebrovascular disease, microalbuminuria independent of well controlled blood sugar levels.^(3,8)

Changes of natural anticoagulants in diabetes

An important aspect of the hemostatic process is the regulation of blood clotting by natural anticoagulants. There are two major groups of natural anticoagulants: 1) serine protease inhibitors [antithrombin (AT), heparin cofactor II and tissue factor pathway inhibitor (TFPI)] and 2) inhibitors of activated protein C (PC) and its cofactor protein S (PS). These mechanisms limit fibrin formation to the area where there is endothelial injury.⁽⁴²⁾

Deficiencies of PC, PS and AT are considered risk factors for thrombotic events⁽⁴⁰⁾ because low plasma levels impair the efficiency of natural anticoagulant mechanisms. Villanueva & Allen demonstrated that hyperglycemia promotes a reduction in the biological activity of AT in diabetic individuals, but that the antigen concentration remains normal or increased. It is speculated that the non-enzymatic glycation of the protein causes structural changes that lead to dysfunction.^(43,44) Diabetic and obese patients have reduced levels of AT, a fact that favors the prothrombotic state.⁽⁴⁵⁾ However, other studies have shown elevated levels of AT in patients with diabetic retinopathy, those with low blood sugar control and in Blacks.⁽⁸⁾ An increase in TFPI has also been described in patients with diabetic nephropathy.⁽⁸⁾

Plasma levels of antigenic and functional PC appear to be high in diabetic patients⁽⁴⁶⁾ and it is known that this increase is not due to inflammatory processes, as PC is not an acute phase protein.⁽⁴⁷⁾ Statin use also influences the PC pathway by increasing the expression of TM in the endothelium and, consequently, increasing PC activation, which results in a potential in vivo antithrombotic effect.⁽³⁷⁾

PC levels can be affected by covariates such as age (older individuals have higher PC concentrations than younger people), pregnancy, menopause, use of hormone replacement therapy in women and oral contraceptives that also increase PC levels.⁽⁴⁸⁾

Quantitative and qualitative deficiencies of PC, PS and AT are well established risk factors for venous

thromboembolism, but elevated levels provide no reduction in the risk of thrombosis and, thus far, have no clinical significance.^(40,48)

Changes in the fibrinolytic system in diabetes

Clots are scheduled to be temporary structures. After the reconstitution of the endothelium, the fibrin clot is broken down by the fibrinolytic system. Plasminogen is converted into plasmin, which in the last stage acts on fibrin leading to the formation of D-dimers (D-Di). The fibrinolytic system is also regulated by activators and inhibitors.⁽⁴⁹⁾

The fibrinolytic system can be inhibited in two distinct ways. The plasminogen activator inhibitor type 1 (PAI-1) inhibits both tissue-type and urokinase-type plasminogen activators (t-PA and u-PA), thereby reducing the generation of plasmin. However, other inhibitors, such as alpha2-antiplasmin, act directly on plasmin, inhibiting its catalytic action.⁽⁵⁰⁾

PAI-1 is synthesized by different sources, such as endothelial cells, adipose tissue and liver cells. Large amounts of PAI-1 are stored in platelets and mediate the formation of platelet-rich clots which are resistant to fibrinolysis. Several inflammatory cytokines such as interleukin 1 (IL-1) and TNF stimulate the endothelial synthesis of PAI-1 as well as growth factors and hormones (estrogen, insulin and thrombin).⁽⁵¹⁾

The levels of PAI-1 increase with advancing age, presenting a mean level of 50 to 60 ng/mL and the concentrations range from undetectable levels to more than 200 ng/mL. This is because there is a circadian variation in which higher levels are observed in the morning. This variation may have a direct influence on the peak incidence of acute myocardial infarction.⁽⁵¹⁾

Hyperglycemia acts on the fibrinolytic system by stimulating PAI-1 production. This condition favors the permanence of the fibrin clot, and consequently the development of thrombi.^(24,49,52)

Some studies have shown that elevated PAI-1 levels are also an independent risk factor in the development of T2DM in healthy subjects, i.e. an increase of PAI-1 precedes T2DM.⁽¹³⁾ Elevated PAI-1 levels are associated with components of insulin resistance syndrome, such as increased body mass index, hypertension, hypertriglyceridemia and hyperinsulinemia in apparently healthy subjects with insulin resistance, in T2DM patients and in patients with cardiovascular disease.^(25,36) Interventional studies have shown that weight loss, exercise and metformin reduce PAI-1 levels.⁽¹³⁾

Additionally, t-PA is increased in diabetic patients, but most of the t-PA circulates bound to PAI-1. Thus, we speculate that the increase in t-PA is due to the increased circulating PAI-1. However the PAI-1 levels exceed t-PA levels thus the increase in t-PA does not benefit individuals

with a greater potential to produce plasmin and fibrin clot lysis.⁽¹³⁾

In many studies, diabetic patients had increased levels of D-Di.^(53,54) However, under normal conditions, when there is a hypercoagulable state, there is consequently a state of hyperfibrinolysis. As hypercoagulability and hypofibrinolysis states are present in diabetic patients, the expression of markers such as Di-D may be underestimated.⁽⁵³⁾ Moreover, one should take into account that the more rigid fibrin from glycosylated fibrinogen may also contribute to relatively low values, as this fibrin is more difficult to break down.

The clinical utility of D-Di is currently limited to excluding deep venous thrombosis and pulmonary thromboembolism from the diagnosis with the finding of low values due to the negative predictive value of this marker. However, we can not ignore the increased levels of D-Di that are not directly associated with deep venous thrombosis and pulmonary thromboembolism.⁽⁵⁴⁾

Final considerations

Much is known about the associations between diabetes and hemostatic markers. However, because diabetes is a multifactorial disease, some discrepancies between studies are observed and thus more detailed studies are necessary to elucidate the process and clarify the factors involved.

It is important to remember that diabetes is not a disease that can only be categorized as "yes" or "no." The degree of impairment, associated diseases and therapeutic measures directly influence disease progression and the levels of laboratory markers evaluated in these individuals.⁽⁶⁾

Laboratory evidence of hemostatic abnormalities in diabetic patients supports the clinical observation that diabetes is a hypercoagulable and hypofibrinolytic state.

Hemostatic system disorders may be simultaneously present (increases in endothelial injury markers such as vWF, which promotes platelet adhesion to the subendothelium; platelet hyperactivity; hypercoagulability with increased thrombin formation; increased fibrinogen levels, which participate in the formation of platelet and fibrin clots; and decreased fibrinolytic activity, with an increase in PAI-1 which results in a longer permanence of the fibrin clot). All factors may contribute to a greater or lesser degree in the high incidence of thrombotic events in diabetic individuals.⁽³⁶⁾

The association of endothelial damage, changes in platelet reactivity, elevated clotting factors, changes in the structure of fibrin and impaired fibrinolysis favor the development of cardiovascular disease and increase the risk of thrombosis.⁽¹³⁾

A major methodological limitation of cross-sectional studies is that these studies show only associations between variables and are not able to demonstrate causal relationships.

Basic research studies are needed to investigate the mechanisms involved in the development of changes caused by hyperglycemia, hypertension and dyslipidemia and the interactions between the different conditions associated with diabetes.

These clinical conditions characterize diabetes as an important risk factor for thrombosis due to hypercoagulable and hypofibrinolytic states. Hypercoagulability markers, such as fibrinopeptide A, prothrombin fragment 1 + 2, thrombin-antithrombin complex, fibrinogen, soluble von Willebrand factor, soluble thrombomodulin, D-dimer, plasminogen activator inhibitor type 1, among others, may be high, a fact that shows hyperactivation of the hemostatic system indicating a favorable condition for the formation of fibrin.^(3,8,14,18,20)

It is important to stress that clinical and medicinal intervention strategies have not been established yet to deal with changes in the hemostatic system as with hypercholesterolemia and inflammatory processes.

Resumo

O diabetes tem adquirido um caráter de epidemia devido ao grande aumento do número de indivíduos acometidos nas últimas décadas. A mortalidade relacionada ao diabetes está associada a eventos trombóticos, principalmente cardiovasculares. De uma maneira geral, pacientes com diabetes apresentam um quadro de hipercoagulabilidade e hipofibrinólise; entretanto, ainda não foram esclarecidos os mecanismos que deflagram as alterações hemostáticas nos indivíduos diabéticos. O objetivo do presente artigo foi abordar as alterações mais frequentes do sistema hemostático descritas na literatura nos indivíduos diabéticos. Os indivíduos diabéticos apresentam alterações endoteliais, plaquetárias, nos fatores de coagulação, nos anticoagulantes naturais e no sistema fibrinolítico, sendo que essas alterações são causadas direta ou indiretamente pela hiperglicemia. Dessa forma, analitos como fator de Von Willebrand, fator VIII, fibrinogênio e D-dímero são marcadores que apresentam interpretações diferenciadas no contexto do paciente diabético. As evidências laboratoriais das alterações hemostáticas nos indivíduos diabéticos suportam a observação clínica de que o diabetes é um estado de hipercoagulabilidade e hipofibrinólise. Ainda não estão bem estabelecidas as estratégias de intervenção clínica e/ou medicamentosa frente aos resultados alterados do sistema hemostático.

Descritores: Diabetes melito tipo 2; Hemostasia; Trombofilia; Fibrinólise

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