REVIEW ARTICLE

PLATELETS STRUCTURAL, FUNCTIONAL AND METABOLIC ALTERATIONS IN DIABETES MELLITUS

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Diabetes mellitus is associated with micro and macrovascular complications. Cardiovascular complications are the major cause of mortality in diabetics. Platelets of patients with diabetes show a large number of structural, functional and metabolic changes. Aim of this review is to discuss all these changes and provides a better understanding of the diseae process.
Keywords: Diabetes mellitus, platelets, thrombosis.

Diabetes mellitus is a prothrombotic state with increased activation of platelets and coagulation proteins and decreased fibrinolytic activity.[1] Diabetic thrombocytopathy refers to differences in platelet functions between diabetic and non diabetic individuals. Platelets from diabetic patients exhibit enhanced platelet aggregation early in the course of the disease that may precede the development of cardiovascular complications.[2] Platelets from diabetic subjects exhibit certain abnormal features which render these individuals more prone to thrombotic episodes.

Enhanced platelets activation

There is evidence for the in vivo activation of circulating platelets in patients with diabetes mellitus.[3] Most reports suggest that there may be a specific priming of hyper sensitive platelets in diabetics in response to platelet agonists. Circulating platelets go through more frequent episodes of release of their granules. Augmented granule release implies reduced platelet survival due to their accelerated sequestration in the circulation. Increased platelet turnover reflects increased thrombopoiesis in diabetic patients.[4,5]

Altered response to agonists, enhanced glycoprotein receptors expression, increase in adhesive proteins on the platelet surface, increased fibrinogen binding and decreased membrane fluidity is instrumental in platelet activation.

Platelets hyperaggregability

Platelets hyper aggregation in response to glucose was recognised in 1965.[6] Enhanced platelet aggregation in response to ADP, thrombin, collagen, arachidonic acid and epinephrine is seen in patients with diabetes mellitus as compared to non-diabetic individuals.[7] Under *in vitro* conditions these platelets after stimulation with platelet agonists show reduced threshold for platelet aggregation.[8]

Increased platelet aggregation is more apparent in patients with diabetes associated with macro vascular disease.[9] Platelets from diabetic subjects show an increased adhesiveness and increased spontaneous aggregation as well as aggregation on extra cellular matrix.[8]

Increased arachidonic acid metabolism

Thromboxane A_2 (TXA_2) is one of the most potent platelet activators. Enhanced activation of arachidonic acid pathway leads to increased TXA_2 formation, increased phosphoinositide turnover resulting in increased protein phosphorylation, enhanced inositol trisphosphate production and subsequently accelerated Ca^{2+} mobilisation.[10]

Increased TXA_2 production in patients with diabetes has been reported in vitro as well as *in vivo*. Addition of platelet agonists to platelet rich plasma resulted in increased TXA_2 synthesis *in vitro*.[11] Increased urinary excretion of 11-dehydro-TxB_2 supports increased thromboxane metabolism *in vivo*.[12,13]

Plasma glucose concentration and HbA1c directly affect thromboxane metabolism. Improved glycaemic control has been shown to reduce thromboxane A_2 production in several[11,13] but not in all studies.[14,15] Increased TXA_2 production has been related to diabetes-associated micro and macro angiopathy.[12,13]

Increased calcium flux

Platelets of patients with type 2 diabetes mellitus show abnormal calcium homeostasis. Increased calcium mobilisation from intra-platelet storage pool and higher levels of intracellular free calcium has been reported in patients with diabetes mellitus.[16,17] Intracellular free calcium has been correlated with the reduction in membrane fluidity.[16] Altered properties of platelet membranes are related to calcium mobilisation resulting in platelet hyper function.[10,16,18] In addition to alterations in platelet calcium homeostasis, intracellular magnesium concentrations are also reduced consistent with an increase in platelet hyperaggregability and adhesiveness.[19]

Platelets nitric oxide synthesis

Nitric oxide (NO) and prostacyclin inhibit platelet-endothelium interactions and promote endothelium mediated vasodilation. Platelets from diabetic patients produce less NO and prostacyclin. Concentration of NO is less in the platelets of diabetic patients than non-diabetic individuals.[20] Insulin stimulates NO synthesis in platelets.[21]

Platelet secretary products

Activated platelets release mitogenic and chemotactic factors like platelet-derived growth factor, transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived epidermal growth factor (PDEGF) and insulin-like growth factor-1 (IGF-1). Platelet factor-4 (PF-4), plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor, β-thromboglobulin, fibrinogen, fibronectin and thrombospondin are also significantly released upon platelet activation.[22] Elevated plasma levels of β-thromboglobulin and PF-4 have been observed in patients with diabetes mellitus.[14,23]

Platelet membrane glycation

Hyperglycaemia is responsible for the non-enzymatic glycation of platelet membrane proteins. Non-enzymatic glycation of platelet membrane proteins leads to alterations in the protein structure, conformation and membrane lipid dynamics.[24–26] Reduced platelet membrane fluidity appears to be related to the extent of glycation of membrane proteins.

Altered dynamics of platelet membrane lipids in turn results in enhanced expression of receptors that are crucial for platelet functions. These include P-selectin, fibrinogen receptors and von Willebrand factor receptors.[27,28] Increased expression of adhesion receptors, e.g., αIIbβ3 have been attributed to more frequent episodes of platelet activation and degranulation. Increased expression of these receptors makes these molecules much more susceptible to potential ligands.[29]

Membrane glycation of low density lipoproteins

Low density lipoproteins (LDL) glycation has been shown to be contributory factor towards increased platelet sensitivity to aggregating agents.[30] Degree of LDL glycation positively correlates with the rate of platelet aggregation.[30] Hyperglycaemia causes an increase in non-enzymatically glycated LDL (glycLDL) rendering the platelets more susceptible to oxidative stress.[31,32] GlycLDL inhibits platelet membrane Ca^{2+}-ATPase which may lead to increased intracellular Ca^{2+} concentration and decreased NO synthase activity.[33]

Inhibition of platelet membrane Na^{+}/K^{+}-adenosine triphosphatase (Na^{+}/K^{+}-ATPase) activity is another mechanism of platelet dysfunction due to glyLDL.[34] Lipoproteins also enhance thromboxane generation during platelet activation.[35] Oxidised lipids provide a surface for the activation of prothrombinase complex in diabetes mellitus.

Expression of increased surface markers on platelet membrane

Platelets from diabetic patients have increased number, adhesiveness and activity of several platelet specific glycoprotein receptors (GP). Increase in the number of GPIIb/IIIa (αIIbβ3),[27] GPIb-IX-V, GPIa/IIa, CD62[36–38] and CD63[38] have been observed in patients with diabetes mellitus. Increased expression of platelet αIIbβ3 is consistent with enhanced fibrinogen binding and aggregability in patients with diabetes.[39] Platelet receptor activation has been correlated with glycaemia in clinical[38] and experimental studies[40] and also with vascular complications.[37]

Enhanced surface expression of these adhesion molecules suggests that platelets also communicate with leukocytes. Platelets play a pivotal role in inflammation mediated tissue damage in the vessels. Up-regulation of CD40-CD40 ligand system has been observed in patients with diabetes mellitus.[41] CD40L on platelets correlates with high HbA1c levels.[41]

P-selectin and CD40L are shed from platelet surface into plasma in biologically active soluble form.[42,43] Studies have shown elevated levels of soluble P-selectin.[44] and CD40L[37,45-47] in patients with diabetes mellitus and cardiovascular diseases.[48] Elevated levels of these compounds may reflect a prothrombotic state and accelerated atherosclerosis.[49–51]

In patients with diabetes mellitus platelets also show hypersensitivity to collagen. Elevated expression of platelet Fc-receptor correlates with increased collagen-induced aggregation.[52,53]

Platelet metabolic alterations

Since glucose entry into the platelets do not depend on insulin, intra-platelet glucose concentration mirrors the extra cellular concentration.[54] Hyperglycaemia has been clearly established as a causal factor for *in vivo* platelet activation and platelet hyperactivity in patients with diabetes mellitus.[55]

Hyperglycaemia induces a number of changes in the platelet functions. It causes increased activation of platelets exposed to high shear stress both *in vitro* and *in vivo*.[56] Metabolic alterations of platelets leading to increased sensitivity to agonists are due to impaired calcium homeostasis, activation of PKC, decreased production of platelet-derived nitric acid and increased formation of superoxide anion. Reduced glutathione levels and nitric oxide synthase activity are some other metabolic alterations in the platelets of patients with diabetes.[54,57,58]

Altered platelet size and volume

Predominantly large platelets circulate in patients with diabetes mellitus. This has been considered secondary to an increased ploidy and activation of megakaryocytes.[59] Larger and younger platelets are considered to be more reactive.

Mean platelet volume (MPV) correlates well with the number of glycoprotein molecules on platelet membrane, the thromboxane synthesising capacity and platelet granule contents of various platelet specific proteins.[4] Increased MPV has also been associated with

proliferative diabetic retinopathy.[4] However, MPV does not seem to be influenced by glycaemic control.[60]

Platelet life span

Studies on platelet survival in patients with diabetes mellitus have produced conflicting results. Some studies have shown decreased platelet survival in patients with diabetes mellitus with overt vascular complications.[61] Other researcher however did not find any difference in platelet survival and vascular complications. They also failed to demonstrate any relationship between platelet survival and vascular complications in patients with diabetes mellitus compared to normal healthy controls.[62,63]

Platelet-leukocyte interaction

Inflammation and thrombosis cause activation of endothelial cells, leukocytes and platelets. Complex interaction between these cells is influenced by several mediators.

Platelets may influence leukocyte activation, chemotaxis and phagocytosis.[64] Platelet-released adenine nucleotides and platelet derived growth factor induce leukocyte degranulation. Adherent platelets, platelet-derived microparticles, PDGF, PF-4 and TXA_2 enhance leukocyte rolling and adhesion.[65] PDGF is also a chemottractant and enhances phagocytosis by neutrophils and monocytes. Superoxide formation by neutrophils may be enhanced by platelets bound to neutrophils or platelet-released ADP while intact non-stimulated platelets may inhibit neutrophil superoxide production.[66] Leukocyte chemotaxis, adhesion and superoxide generation are inhibited by P-selectin and nitric oxide released from platelets.[67]

Platelets and platelet-derived products influence leukocyte function in several ways. Platelets and leukocytes may form platelet-leukocyte aggregates or conjugates (PLAs) mainly via platelet-expressed P-selectin and its receptors P-selectin glycoprotein ligand-1, CD15, αIIbβ3 and CD11b/CD18.

Diabetic angiopathy involves atherosclerosis, inflammation and thrombosis due to abnormal function of platelet and leukocyte. In diabetic microangiopathy increased platelet and leukocyte activation and heterotypic aggregation are evident.[68]

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