Review Article:

Molecular mechanisms underlying microvascular complications in diabetes mellitus

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ABSTRACT

Vascular complications are a major cause of morbidity and mortality in patients with diabetes mellitus. Diabetic microvascular complications include diabetic retinopathy, neuropathy and nephropathy. Hyperglycaemia-induced activation of metabolic pathways, hyperglycaemia-induced growth factors, components of metabolic syndrome and hyperglycaemia-induced epigenetic changes act through a common platform i.e endothelial dysfunction. Hyperglycaemia, is the initiating cause of diabetic tissue damage. Under conditions of hyperglycaemia, four important pathways are activated which shuttle glucose and its intermediates through alternate pathways especially the polyol pathway forming sorbitol which exerts an osmotic effect; advanced glycation end products which modify biomolecules and alter their functions; protein kinase C activation causing altered signal transduction and hexosamine pathway which forms uridine diphosphate) N-acetyl glucosamine which glycosylates transcription factors and increases expression of procoagulant molecules. Hyperglycaemia is thought to activate these four pathways through increased generation of superoxide anions. Though hyperglycaemia is thought to be essential to cause clinically important microangiopathy, there are other factors which predispose an individual to these complications. Hyperglycaemia-induced epigenetic changes i.e., changes in the deoxyribonucleic acid (DNA) molecule due to causes outside the DNA molecule are currently being probed for their role in development and progression of vascular complications. The ultimate purpose of understanding these mechanisms is to devise therapeutic measures which will target these mechanisms and will help in preventing the development as well as delaying the progression of diabetic vascular complications and improve the quality of life in these patients.

Key words: *Diabetes mellitus, Endothelial dysfunction, Microvascular; Hyperglycaemia, Epigenetics*

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both.[1] The chronic hyperglycaemia of diabetes affects the vascular system directly or indirectly and is the main cause of morbidity and mortality seen in these patients.[2] These injurious effects are broadly classified into the macrovascular and the microvascular complications.[2]

Macrovascular complications mainly involve the large vessels such as arteries and veins clinically manifesting as cardiovascular disease, peripheral artery disease and cerebrovascular disease.[2] Cardiovascular disease (CVD) is the leading cause of death (~70%) in patients with type 2 DM (T2DM).[3,4] Although, CVD risk factors like age, obesity, dyslipidaemia, hypertension are more common in these patients, diabetes itself is an independent risk factor for CVD.[5] Similarly, diabetes is an independent risk factor for stroke across all ages.[6] Presence of DM adversely affects the cerebrovascular circulation by increasing the risk of intracranial and extra-cranial (e.g., carotid artery) atherosclerosis.[3,7]

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Peripheral artery disease characterized by occlusion of the lower extremity arteries,[8] can result in functional impairment and disability, and is related to the duration and severity of diabetes.[9]

Microvascular complications involve the small vessels such as the capillaries and include diabetic retinopathy, neuropathy and nephropathy affecting the vessels of the retina, nerves and the kidneys respectively. Diabetic retinopathy (DR) is a complication affecting the peripheral retina, the macula, or both and is a leading cause of visual disability and blindness in people with diabetes. The severity of DR ranges from the less severe nonproliferative and preproliferative to the more severe proliferative DR, characterized by abnormal growth of new vessels.[10] About 50% of patients with DM can develop either autonomic or peripheral neuropathy. Autonomic neuropathy manifests as abnormal heart rate and vascular control.[11] Characteristic features of peripheral neuropathy include axonal thickening with progression to axonal loss, basement membrane thickening, pericyte loss, loss of microfilaments (actin and myosin), and decreased capillary blood flow to C fibers, leading to decreased nerve perfusion and endoneurial hypoxia.[3] Diabetic nephropathy (DN) is a serious and progressive complication which typically manifests as microalbuminuria. Other characteristic features of DN include thickening of glomerular basement membranes and glomerular hyperfiltration, leading to mesangial extracellular matrix expansion. This leads to further increase in urinary albumin excretion and progression to glomerular and tubular sclerosis and renal failure.[3]

The principle biochemical mechanisms involved in these complications include the hyperglycaemia induced activation of the metabolic pathways[12-14], oxidative stress[15] and growth factors (Figure 1). Endothelial dysfunction is being increasingly recognized as the principle mediator of both micro-and macrovascular complications in diabetes.[16]

Dysfunction of the vascular endothelium is regarded as an important factor in the pathogenesis of micro- and macro-angiopathy. Endothelial dysfunction is an early reversible step in the process of atherosclerosis.[17] Under normal conditions, the vascular endothelium releases vasoprotective factors like nitric oxide (NO) also called the endothelium-derived relaxing factor, prostacyclin, bradykinin, and endothelium-derived hyperpolarizing factor (EDHF) and harmful vasoconstrictor substances like endothelin (ET), reactive oxygen species (ROS), endothelium-derived cyclooxygenase (COX)-dependent vasoconstricting factor (EDCF), and angiotensin II (Ang II) to maintain vascular homeostasis with a anticoagulant, antithrombotic and fibrinolytic phenotype. Nitric oxide is synthesized by endothelial nitric oxide synthase (eNOS) and is the chief vasodilator. Damage to the endothelial layer upsets the balance between vasoconstriction and vasodilation, initiating a number of events/processes that promote or exacerbate atherosclerosis[18] via increased endothelial permeabilization, platelet aggregation, leukocyte adhesion, and cytokine production.

MICROVASCULAR COMPLICATIONS MECHANISMS

The present review discusses the mechanisms involved in diabetic microvascular complications and its clinical implications.

Endothelial dysfunction in diabetic microvascular Complications has been attributed to the following hyperglycaemia linked mechanisms.[19] (i) hyperglycaemia-induced activation of biochemical pathways; (ii) hyperglycaemia-induced synthesis of growth factors and vasoactive agents; (iii) components of metabolic syndrome and (iv) hyperglycaemia-induced epigenetic changes.

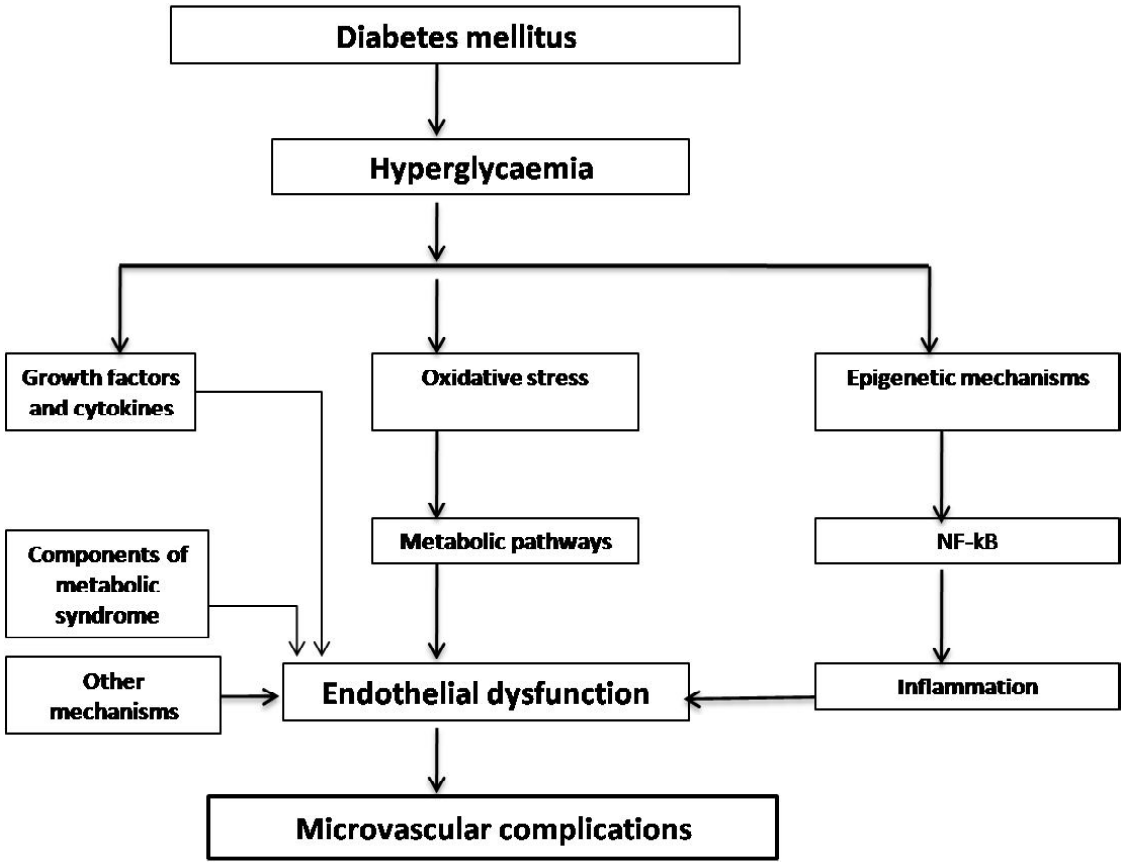

Figure 1: Molecular mechanisms underlying diabetic microvascular complications
NF-KB=nuclear factor kappa beta

Hyperglycaemia-induced activation of biochemical pathways

The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) established that hyperglycaemia, is the initiating cause of the diabetic tissue damage.[20,21] This process is modified by both genetic determinants of individual susceptibility, and by independent accelerating factors such as hypertension, hyperlipidaemia etc. Diabetes selectively damages cells, like endothelial cells and mesangial cells, whose glucose transport rate does not decline rapidly as a result of hyperglycaemia, leading to high glucose inside the cell.[22] Under conditions of hyperglycaemia, four important pathways are activated which shuttle glucose and its intermediates through alternate pathways (Figure 1).[23-26] Hyperglycaemia is thought to activate these four pathways through increased generation of superoxide anions.[15]

Under normal conditions, glucose after glycolysis and the Krebs cycle generates reducing equivalents in the form of reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$). The reducing equivalents then enter the electron transport chain located on the inner side of the inner mitochondrial membrane to donate the reducing equivalents to molecular oxygen and in the process leads to synthesis of adenosine tri phosphate (ATP). However, under conditions of hyperglycaemia, increased flux of reducing equivalents through the ETC causes the voltage gradient across the mitochondrial membrane to increase until a critical threshold is reached. When this is achieved, electron transfer inside complex III is blocked causing the electrons to back up to coenzyme Q, which

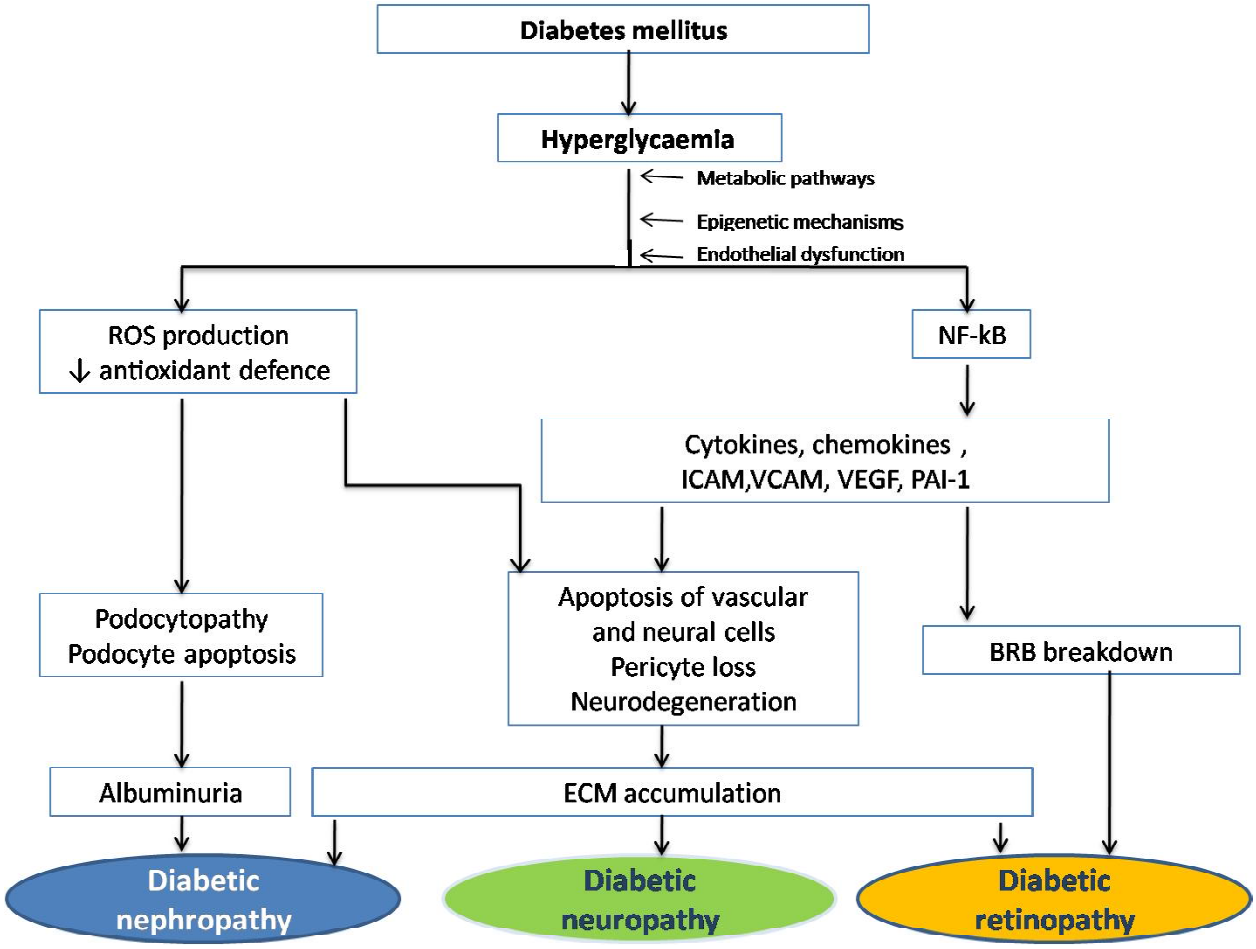

Figure 2: Pathways leading to development of microvascular complications
NF-KB= nuclear factor kappa beta; ROS= reactive oxygen species; ECM= extracellular matrix; ICAM= intercellular adhesion molecules; VCAM= vascular cell adhesion molecule; VEGF= Vascular endothelial growth factor; PAI= plasminogen activator inhibitor; BRB= blood retinal barrier

donates electrons one at a time to molecular oxygen, thereby generating superoxide.[27] It has been proposed that hyperglycaemia-induced mitochondrial superoxide production activates the four damaging pathways by inhibiting glyceraldehydes-3 phosphate dehydrogenase (GAPDH) through the activation of poly adenosine diphosphate (ADP) ribose polymerase.[15] The four pathways are the polyol pathway, glyoxylation pathway, protein kinase C pathway and the hexosamine pathway.

Polyol pathway

The first pathway proposed was the polyol pathway. Under normal conditions, aldose reductase (AR) reduces toxic aldehydes in the cell to inactive alcohols, but under conditions of hyperglycaemia, AR also reduces glucose to sorbitol. This sorbitol exerts osmotic effect and thereby causes damage to cells. Sorbitol is later oxidized to fructose in a reaction consuming the cofactor reduced nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is an essential cofactor for regenerating reduced glutathione, an important intracellular antioxidant protecting cell against reactive oxygen species (ROS). Glutathione exists in the oxidized glutathione (GSSG) (inactive) form and the reduced (GSH)(active)form. Depletion of NADPH in the polyol pathway thus increases the susceptibility of cells to intracellular oxidative stress.[28]

Studies have shown that AR catalyzes the reduction of a large number of aldehydes

generated from lipid peroxidation with 10^3 to 10^4 fold higher efficiency than glucose and that glucose may be an incidental substrate of AR.[29] AR is susceptible to oxidants such as hydrogen peroxide (H_2O_2) and NO owing to the presence of a highly reactive cysteine (Cys-298) residue at its active site. These oxidants can cause inactivation of AR. The enzyme also catalyzes the reduction of the glutathione conjugates of unsaturated aldehydes, which is considered to be a protective mechanism in minimizing the reactivity of the aldehyde function unquenched by glutathiolation. AR can be inactivated by undergoing glutathiolation of the Cys- 298 residue.[30]

NO has been shown to regulate intracellular AR activity.[31] AR is either S-thiolated (inactivated enzyme) or S-nitrosated (activated enzyme) depending on the conditions of the reaction and the nature of the NO donor used. NO secreted by endothelial cells can undergo S-glutathionylation and form GSNO with glutathione that is abundant in vascular smooth muscle cells (VSMC). The GSNO formed can in turn S-glutathiolate AR at Cys-298 [32] S-glutathiolation inactivates AR and thus under normoglycaemic conditions, much of the AR is in the inactive form, whereas in hyperglycaemia a decrease in NADPH/NADP+ ratio and other factors decrease NO and the AR would be in the active form. S-glutathiolation of AR in vascular smooth muscle cells (VSMC) by NO donors such as GSNO, which inactivates the enzyme, is reversible.

AR can thus be a therapeutic target for preventing or treating diabetic complications through increasing NO levels.[33,34] AR is also a critical component of intracellular signaling, and inhibition of the enzyme prevents high glucose-, cytokine-, or growth factor-induced activation of protein kinase C and nuclear factor-kappa beta (NF-Kβ)-binding protein.[35]

Glyoxylation pathway

The second pathway is the glyoxylation pathway which increases the intracellular concentration of advanced glycation end products (AGEs).[36] AGEs are heterogeneous group of molecules formed from the non enzymatic reaction of reducing sugars with the free amino groups of proteins, lipids and nucleic acids. AGE precursors diffuse out of the cell and bind covalently with proteins forming crosslink's thus modifying circulating proteins in the blood[37] such as albumin. These modified circulating proteins can then bind to AGE receptors (RAGEs)[38] and are taken up by endocytosis.

Endocytosed AGEs activate the cells causing the production of inflammatory cytokines and growth factors, which in turn have been implicated in vascular pathology.[39,40] AGEs also directly affect the vascular endothelium. Diabetic patients have been shown to have impaired endothelial-dependent and endothelium-independent vasodilatation which correlated with serum AGEs concentration.[41] Depletion of endothelial NO production was augmented by AGEs[42] thus showing the direct effects of AGEs on endothelial cells apart from causing the inflammatory response.

Protein kinase C pathway

The third pathway proposed is the activation of protein kinase C (PKC) pathway.[43] PKC phosphorylates a number of proteins and regulates their activity. Hyperglycaemia inside the cell increases the synthesis diacylglycerol, a critical activating cofactor for the classic isoforms of PKC, β, δ, and α. High glucose, via PKC signaling affects gene expression of a number of important molecules thereby leading to decreased synthesis of endothelial nitric oxide (NO) synthase (eNOS).[44] It also induces oxidative stress and up-regulation of COX-2, resulting in reduced NO availability and altered prostanoid profile.[45] PKC activation has been

shown to produce endothelium-dependent vasodilator dysfunction by altering the bioavailability of nitric oxide (NO), and increasing the production of thromboxane, other COX-dependent vasoconstrictors and endothelin-1 (ET-1),[46] causing an imbalance in the vasodilating nitric oxide and the vasoconstricting endothelin-1.

Hexosamine pathway

The fourth pathway activated in the presence of hyperglycaemia is the hexoamine pathway. In glycolysis, glucose is first converted to glucose-6 phosphate and then fructose-6 phosphate followed by the further reactions that lead to the formation of pyruvate under aerobic conditions. However, under conditions of hyperglycaemia, some of the fructose-6-phosphate gets diverted into a signalling pathway in which an enzyme glutamine: fructose-6 phosphate amidotransferase (GFAT) converts fructose-6 phosphate to glucosamine-6 phosphate and finally to uridine diphosphate (UDP) *N*-acetyl glucosamine (UDP-GlcNAC).[47]

UDP-GlcNAC the product of activation of the hexosamine pathway glycosylates transcription factors on the serine and threonine residues leading to altered gene expression. Eg. modification of transcription factor Stimulatory protein (Sp1) results in increased expression of transforming growth factor-β and plasminogen activator inhibitor-1 (PAI-1) which leads to a procoagulant state, modification of eNOS leading to decreased NO synthesis. O-GlcNAcylation also impairs the activation of the insulin receptor signalling pathway, resulting in deregulation of eNOS activity and decreased NO production.[48]

Hyperglycaemia-induced synthesis of growth factors and vasoactive agents

Hyperglycaemia-induced synthesis of growth factors, cytokines and vasoactive agents in different cells can indirectly affect endothelial cell functioning.[49] Vascular endothelial growth factor (VEGF) and the angiopoietins are two families of growth factors that act on vascular endothelial cells and are involved in angiogenesis.[50] During angiogenesis, VEGF interacts with several other angiogenic factors, playing an important role in cell proliferation, differentiation, migration, cell survival, NO production, release of other growth factors, and sympathetic innervation.[51] Diabetic patients have been shown to have elevated circulating levels of VEGF.[52] Under normal physiological conditions VEGF-A is constitutively expressed in the glomerular podocytes. Up-regulation of VEGF-A in early diabetic nephropathy has been reported.[52] This leads to glomerular capillary pathology and increased vascular permeability. Experimental studies have shown that up-regulation of VEGF leads to activation of transforming growth factor (TGF)-β1, and stimulates extracellular matrix production and accumulation leading to glomerular basement membrane thickening.[53] A complex interaction exists between VEGF-A, NO and endothelial dysfunction in diabetic nephropathy. VEGF is beneficial for endothelial cells when NO is synthesized normally. Conditions which lead to decrease in NO synthesis/bioavailability cause the deleterious effects of VEGF to predominate.[54] Interaction of VEGF with endothelium leads to alteration in retinal microvasculature and increased vascular permeability contributing to diabetic retinopathy.[55] VEGF is thus an important therapeutic target in diabetic retinopathy.[56]

The inflammatory cytokine tumour necrosis factor- α (TNF-α), produces endothelial dysfunction directly or through the production of interleukin-6 (IL-6) and the downstream C-reactive protein (CRP) by the liver. These factors independently or in combination can lead to endothelial dysfunction.[49] Hyperglycaemia and TNF- α also cause activation of NF-KB which leads to activation of a number of genes involved in acute

inflammatory response.[57,58] NF-KB regulates the expression of a number of genes, including the expression of adhesion molecules intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, interleukin (IL-1), interleukin-6 (IL-6) and interleukin-8) (IL-8) tissue factor, PAI-1 and inducible NOS (Figure 2).[49,58] These markers were found to be elevated even in normoalbuminuric patients, indicating the early occurrence of vascular dysfunction even before it manifests clinically as microalbuminuria.[59]

Apart from causing endothelial dysfunction they also produce other effects like increasing the vascular permeability, alteration of vasoregulatory responses, increasing leucocyte adhesion to endothelium and facilitate thrombus formation by inducing pro-coagulant activity and by inhibiting anti-coagulant pathways by impairing fibrinolysis via stimulation of PAI-1.[49,58]

Components of metabolic syndrome

Metabolic syndrome is a clustering of several risk factors including diabetes and raised fasting plasma glucose, abdominal obesity, high cholesterol and high blood pressure.[60] All elements of the syndrome share two pathophysiological features i.e insulin resistance and endothelial dysfunction.[61]

Hyperglycaemia and elevated free fatty acids seen in diabetes have been shown to up-regulate Bone morphogenic protein 4 (BMP4) in endothelial cells.[62] BMP4 has been shown to induce an inflammatory response following oscillatory shear stress leading to increased expression of intracellular adhesion molecule-1 (ICAM-1) and monocyte adhesion.[63] BMP4 infusion was shown to impair endothelium-dependent dilatation of mouse arteries and increase the vascular NADPH oxidases activity. BMP4 was also reported to impair endothelium-dependent vasodilatation through oxidative stress–dependent and COX-2–dependent mechanisms.[64,65] BMP4 may thus serve as one of common initiators of endothelial dysfunction in hypertension and diabetes through increasing oxidative stress within the vascular wall.

Obesity contributes to endothelial dysfunction through insulin resistance as well as the effects of adipose tissue derived hormones i.e adipokines and pro-inflammatory cytokines which can induce oxidative stress and thus reduce the bio-availability of NO.[65] Obese individuals also have elevated levels of endothelins and PAI.[66]

Hyperglycaemia-induced epigenetic changes

Another aspect of diabetic vascular complications which is currently being probed is the epigenetic mechanisms which deal with changes in the DNA molecule due to causes outside the DNA molecule i.e changes in the cellular environment. An individual's risk of developing microvascular complications is also dependent on the complex interaction between genetic factors and environmental interactions, especially dietary habits and lifestyle, which accelerate or slow down the disease progression. These environmental factors mainly trigger an inflammatory response thereby promoting inflammation-mediated insulin resistance and endothelial dysfunction.[67] It has been shown that transient hyperglycemia induces the so called 'metabolic memory'[68] which promotes gene-activating epigenetic changes and signalling events which are critical in development and progression of vascular complications. Mitochondrial ROS production as a result of hyperglycaemia is said to be the most relevant nuclear epigenetic mechanism. These nuclear epigenetic changes include, DNA methylation and post-translational histone modifications (PTHMs) thereby affecting the chromatin structure. The PTHM changes in diabetes include acetylation of lysine and arginine residues by histone acetyltransferases (HATs), favouring the

euchromatin structure and thus gene transcription to occur.[69] These can become irreversible overtime and thus explain the long-term effects of metabolic memory on vascular complications despite an improvement in glycaemic control.[67]

Cellular events involved in metabolic memory include increased production of AGEs,RAGE overexpression, increased anion superoxide formation, mitochondrial (mt) protein glycation, mt DNA damage, PKC activation, polyol pathway and hexosamine flux alterations.[70] Pro-inflammatory stimuli, including hyperglycaemia, oxidative stress, and other inflammatory mediators, can affect epigenetic mechanisms, altering the expression of specific genes in target cells.[67]

The role of non-coding ribonucleic acids (RNAs) in modulating metabolic memory and thus the risk of developing microvascular complications is emerging. These micro-RNAs (miRs) are a family of small, non-coding RNAs about 21-25 nucleotides in length, that regulate gene expression in a sequence-specific manner. They belong to a novel class of endogenous interfering RNAs that play a crucial role in post transcriptional gene silencing through messenger RNA (mRNA) targeting and are thus involved in many biological processes like apoptosis, cell cycle control, cell proliferation, DNA repair, immunity, metabolism etc.[70]

Although most miRs are involved in gene silencing, a few can also cause gene activation.[71] miRNAs are released into the circulation from cells and can thus serve as biomarkers. Dysregulation of miR expression has been shown in patients in many diseases including T2DM. Abnormal miR levels miR-25 and miR21 have been shown in pre-clinical models of diabetic nephropathy.[72,73] The miR-25 was shown to negatively regulate NOX4 expression, a major catalytic subunit of NADPH oxidase under hyperglycaemia. Recently miR-15a expression levels were shown to be significantly lower in patients with T2DM and impaired fasting glucose /impaired glucose tolerance and could thus serve as a potential biomarker for T2DM and pre-diabetes.[74] In an experimental model, elevated glucose concentration (25 mmol/L) decreased the miR-146a expression and increased expression of extracellular matrix protein, fibronectin; a characteristic of diabetic vascular complications.[75]

Thus, multiple mechanisms act in consert through a common platform i.e., by causing endothelial dysfunction, the basic mechanism triggering microvascular complications. The ultimate purpose of understanding these mechanisms is to devise therapeutic measures which will target these mechanisms and will help in preventing the development as well as delaying the progression of diabetic vascular complications and improve the quality of life in these patients.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2004;27 Suppl 1:S5-S10.
2. Fowler MJ. Microvascular and Macrovascular Complications of Diabetes. Clinical Diabetes 2008;26:277-82.
3. Cade WT. Diabetes-Related Microvascular and Macrovascular Diseases in the Physical Therapy Setting. Phys Ther 2008; 88:1322-35.
4. Laakso M. Hyperglycemia and cardiovascular disease in type 2 diabetes. Diabetes. 1999;48:937-42.
5. Buyken AE, von Eckardstein A, Schulte H, Cullen P, Assmann G . Type 2 diabetes mellitus and risk of coronary heart disease: results of the 10-year follow-up of the PROCAM study. Eur J Cardiovasc Prev Rehabil 2007;14:230-6.
6. Abbott RD, Curb JD, Rodriguez BL, Masaki KH, Popper JS, Ross GW, Petrovitch H. Age-related changes in risk factor effects on the incidence of thromboembolic and hemorrhagic stroke. J Clin Epidemiol 2003; 56:479-86.
7. Friedlander AH, Maeder LA. The prevalence of calcified carotid artery atheromas on the

panoramic radiographs of patients with type 2 diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000;89:420-4.

8. Kullo IJ, Bailey KR, Kardia SL, Mosley TH Jr, Boerwinkle E, Turner ST. Ethnic differences in peripheral arterial disease in the NHLBI Genetic Epidemiology Network of Arteriopathy (GENOA) study. Vasc Med 2003;8:237-42.
9. Jude EB, Oyibo SO, Chalmers N, Boulton AJ. Peripheral arterial disease in diabetic and nondiabetic patients: a comparison of severity and outcome. Diabetes Care 2001; 24:1433-7.
10. Harding S. Extracts from "concise clinical evidence". Diabetic retinopathy. BMJ 2003; 326:1023-5.
11. Vinik AI, Maser RE, Mitchell BD, Freeman R. Diabetic autonomic neuropathy Diabetes Care 2003; 26:1553-79.
12. Pirart J. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973. Diabetes Care 1978,1:168-88.
13. Diabetes Control and Complications Trial: Are continuing studies of metabolic control and microvascular complications in insulin-dependent diabetes mellitus justified? The Diabetes Control and Complications Trial. N Engl J Med 1988;318:246-50.
14. Merimee TJ. Diabetic retinopathy. N Engl J Med 1990;322:978-83.
15. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res 2010;107:1058-70.
16. De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. Br J Pharmacol. 2000;130:963-74.
17. Brunner H, Cockcroft JR, Deanfield J, Donald A, Ferrannini E, Halcox J, et al. Endothelial function and dysfunction, part II: association with cardiovascular risk factors and diseases: a statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. J Hypertens 2005;23:23-46.
18. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004;109:III27-32.
19. Schalkwijk CG, Stehouwer CD. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci; 2005;109:143-59.
20. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-86.
21. UK Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837-53.
22. Brownlee M. The pathobiology of diabetic complications:a unifying mechanism. Diabetes 2005;54;1615-25.
23. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. N Engl J Med 1988;318:1315-21.
24. Wolf BA, Williamson JR, Easom RA, Chang K, Sherman WR, Turk J. Diacylglycerol accumulation and microvascular abnormalities induced by elevated glucose levels. J Clin Invest 1991;87:31-8.
25. Greene DA, Lattimer SA, Sima AA. Sorbitol, phosphoinositides, and sodium-potassium-ATPase in the pathogenesis of diabetic complications. N Engl J Med1987; 316:599-606.
26. King GL, Johnson S, Wu G. Possible growth modulators involved in the pathogenesis of diabetic proliferative retinopathy. In: Growth factors in health and disease. New York: Excerpta Med; 1990.p.303-17.
27. Trumpower BL. The proton motive Q cycle: energy transduction by coupling of proton translocation to electron transfer by the cytochrome bc1 complex. J Biol Chem 1990;265:11409-12.
28. Lee AY, Chung SS: Contributions of polyol pathway to oxidative stress in diabetic cataract. FASEB J 1999;13:23-30.
29. Srivastava S, Watowich SJ, Petrash JM, Srivastava SK, Bhatnagar A. Structural and kinetic determinants of aldehyde reduction by aldose reductase. Biochemistry 1999;38:42-54.
30. Petrash JM, Harter TM, Devine CS, Olins PO, Bhatnagar A, Liu S, et al. Involvement of cysteine

residues in catalysis and inhibition of human aldose reductase. Site-directed mutagenesis of Cys-80, -298, and -303. J Biol Chem 1992;267:24833-40.

31. Chandra D, Jackson EB, Ramana KV, Kelley R, Srivastava SK, Bhatnagar A. Nitric oxide prevents aldose reductase activation and sorbitol accumulation during diabetes. Diabetes 2002;51:3095-101.
32. Chandra A, Srivastava S, Petrash JM, Bhatnagar A, Srivastava SK. Active site modification of aldose reductase by nitric oxidedonors. Biochim Biophys Acta 1997;1341:217-22.
33. Srivastava SK, Ramana KV, Chandra D, Srivastava S, Bhatnagar A. Regulation of aldose reductase and the polyol pathway activity by nitric oxide. Chem Biol Interact 2003;143-144:333-40.
34. Ramana KV, Chandra D, Srivastava S, Bhatnagar A, Srivastava SK. Nitric oxide regulates the polyol pathway of glucose metabolism in vascular smooth muscle cells. FASEB J 2003;17:417-25.
35. Srivastava SK, Ramana KV, Bhatnagar A. Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. Endocrine Reviews 26:380-92.
36. Giardino I, Edelstein D, Brownlee M: Nonenzymatic glycosylation in vitro and in bovine endothelial cells alters basic fibroblast growth factor activity: a model for intracellular glycosylation in diabetes. J Clin Invest 1994;94:110-1.
37. McLellan AC, Thornalley PJ, Benn J, Sonksen PH. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. Clin Sci (Lond) 1994;87:21-9.
38. Neeper M, Schmidt AM, Brett J, Yan SD, Wang F, Pan YC, et al. Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. J Biol Chem 1992;267:14998-15004.
39. Doi T, Vlassara H, Kirstein M, Yamada Y, Striker GE, Striker LJ. Receptor specific increase in extracellular matrix production in mouse mesangial cells by advanced glycosylation end products is mediated via platelet derived growth factor. Proc Natl Acad Sci USA1992;89:2873-77.
40. Schmidt AM, Hori O, Chen JX, Li JF, Crandall J, Zhang J, Cao R, Yan SD, Brett J, Stern D: Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule- 1 (VCAM-1) in cultured human endothelial cells and in mice: a potential mechanism for the accelerated vasculopathy of diabetes. J Clin Invest 1995;96:1395-1403.
41. Tan KC, Chow WS, Ai VH, Metz C, Bucala R, Lam KS. Advanced glycation end products and endothelial dysfunction in type 2 diabetes. Diabetes Care 2002;25:1055-9
42. Soro-Paavonen A, Zhang WZ, Venardos K, Coughlan MT, Harris E, Tong DC, et al. Advanced glycation end-products induce vascular dysfunction via resistance to nitric oxide and suppression of endothelial nitric oxide synthase. J Hypertens 2010;28:780-8.
43. Koya D, King GL: Protein kinase C activation and the development of diabetic complications. Diabetes 1998;47:859-66.
44. Kuboki K, Jiang ZY, Takahara N, Ha SW, Igarashi M, Yamauchi T, et al. Regulation of endothelial constitutive nitric oxide synthase gene expression in endothelial cells and in vivo : a specific vascular action of insulin. Circulation 2000;101:676-81.
45. Cosentino F, Eto M, De Paolis P, van der Loo B, Bachschmid M, Ullrich V, et al. High glucose causes upregulation of cyclooxygenase-2 and alters prostanoid profile in human endothelial cells: role of protein kinase C and reactive oxygen species. Circulation 2003; 107:1017-23.
46. Cardillo C, Campia U, Bryant MB, Panza JA. Increased activity of endogenous endothelin in patients with type 2 diabetes mellitus. Circulation 2002;106:1783-87.
47. Nerlich AG, Sauer U, Kolm-Litty V, Wagner E, Koch M, Schleicher ED. Expression of glutamine:fructose-6-phosphate amidotransferase in human tissues: evidence for high variability and distinct regulation in diabetes. Diabetes 1998;47:170-8.
48. Federici M, Menghini R, Mauriello A, Hribal ML, Ferrelli F, Lauro D, et al. Insulin-dependent activation of endothelial nitric oxide synthase is impaired by O-linked glycosylation modification of signaling proteins in human coronary endothelial cells. Circulation 2002;106:466-72.
49. Schalkwijk CG, Stehouwer CD. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci; 2005;109:143-59.

50. Lim HS, Blann AD, Chong AY, Freestone B, Lip GY. Plasma vascular endothelial growth factor, angiopoietin-1, and angiopoietin-2 in diabetes: implications for cardiovascular risk and effects of multifactorial intervention. Diabetes Care 2004;27:2918-24.

51. Jeong IK, King GL. New perspectives on diabetic vascular complications: the loss of endogenous protective factors induced by hyperglycemia. Diabetes Metab J 2011;35:8-11.

52. Chen S, Ziyadeh FN. Vascular endothelial growth factor and diabetic nephropathy. Curr Diab Rep 2008;8:470-76.

53. Ku CH, White KE, Dei Cas A, Hayward A, Webster Z, Bilous R, et al. Inducible overexpression of sFlt-1 in podocytes ameliorates glomerulopathy in diabetic mice. Diabetes 2008;57:2824-33.

54. Nakagawa T. Uncoupling of the VEGF endothelial nitric oxide axis in diabetic nephropathy: an explanation for the paradoxical effects of VEGF in renal disease. Am J Physiol Renal Physiol 2007;292:F1665–F1672.

55. Fong D S, Aiello LP, Ferris III FL, Klein R. Diabetic retinopathy. Diabetes Care 2004;27:2540-53.

56. Simó R, Sundstrom JM, Antonetti DA. Ocular Anti-VEGF therapy for diabetic retinopathy: the role of VEGF in the pathogenesis of diabetic retinopathy. Diabetes Care 2014;37:893-9.

57. Blann AD, Belgore FM, McCollum CN, Silverman S, Lip PL, Lip GY. Vascular endothelial growth factor and its receptor, Flt-1, in the plasma of patients with coronary or peripheral atherosclerosis, or type II diabetes. Clin Sci (Lond) 2002;102:187-94.

58. Karatzis EM. The role of inflammatory agents in endothelial function and their contribution to atherosclerosis. Hellenic J Cardiol 2005; 46:232-9.

59. Monaco C, Paleolog E. Nuclear factor κB: a potential therapeutic target in atherosclerosis and thrombosis. Cardiovasc Res 2004;61:671-82.

60. Lim SC, Caballero AE, Smakowski P, LoGerfo FW, Horton ES, Veves A. Soluble intercellular adhesion molecule, vascular cell adhesion molecule, and impaired microvascular reactivity are early markers of vasculopathy in type 2 diabetic individuals without microalbuminuria. Diabetes Care 1999;22:1865-70.

61. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C; American Heart Association; National Heart, Lung, and Blood Institute. Definition of Metabolic Syndrome: Report of the National Heart, Lung, and Blood Institute/ American Heart Association Conference on Scientific Issues Related to Definition. Circulation 2004;109:433-8.

62. Bostrom KI, Jumabay M, Matveyenko A, Nicholas SB, Yao Y. Activation of vascular bone morphogenetic protein signaling in diabetes mellitus. Circ Res 2011;108:446-57.

63. Sorescu GP, Song H, Tressel SL, Hwang J, Dikalov S, Smith DA, et al. Bone morphogenic protein 4 produced in endothelial cells by oscillatory shear stress induces monocyte adhesion by stimulating reactive oxygen species production from a NOX1-based NADPH oxidase. Circ Res 2004; 95:773-9.

64. Youn JY, Zhou J, Cai H. Bone Morphogenic Protein 4 Mediates NOX1-Dependent eNOS Uncoupling, Endothelial Dysfunction, and COX2 Induction in Type 2 Diabetes Mellitus. Mol Endocrinol 2015;29:1123-33.

65. Taddei S, Ghiadoni L, Salvetti G, Virdis A, Salvetti A. Obesity and endothelial dysfunction. G Ital Cardiol (Rome) 2006;7:715-23.

66. Calles-Escandon J, Cipolla M. Diabetes and endothelial dysfunction: a clinical perspective. Endocr Rev 2001;22:36-52.

67. Prattichizzo F, Giuliani A, Ceka A, Rippo MR, Bonfigli AR, Testa R, et al. Epigenetic mechanisms of endothelial dysfunction in type 2 diabetes. Clin Epigenetics 2015;7:56.

68. Roy S, Sala R, Cagliero E, Lorenzi M. Overexpression of fibronectin induced by diabetes or high glucose: phenomenon with a memory. Proc Natl Acad Sci USA 1990;87:404-8.

69. Wegner M, Neddermann D, Piorunska-stolzmann M, Jagodzinski PP. Role of epigenetic mechanisms in the development of chronic complications of diabetes. Diabetes Res Clin Pract 2014; 105:164-75.

70. Ceriello A. The emerging challenge in diabetes: the “metabolic memory”. Vascul Pharmacol 2012;57:133-8.

71. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281-97.

72. Vasudevan S, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. Science 2007;318:1931-4.

73. Fu Y, Zhang Y, Wang Z, Wang L, Wei X, Zhang B, et al. Regulation of NADPH oxidase activity is associated with miRNA-25-mediated NOX4 expression in experimental diabetic nephropathy. Am J Nephrol 2010;32:581-9.

74. Al-Kafaji G, Al-Mahroos G, Alsayed NA, Hasan ZA, Nawaz S, Bakhiet M. Peripheral blood microRNA-15a is a potential biomarker for type 2 diabetes mellitus and pre-diabetes. Mol Med Rep 2015;12:7485-90.

75. Feng B, Chen S, McArthur K, Wu Y, Sen S, Ding Q, et al. miR-146a-Mediated extracellular matrix protein production in chronic diabetes complications. Diabetes 2011;60:2975-84.