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COMPLICATIONS OF DIABETES MELLITUS: AN INSIGHT IN TO BIOCHEMICAL BASIS

Bunza J. M.[1]* and Alhassan A. J.[2]

[1]Department of Chemical Pathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

[2]Department of Biochemistry, Faculty of Basic Medical Sciences Bayero University, Kano, Nigeria.

***Corresponding Author: Dr. Bunza J. M.**

Department of Chemical Pathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

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ABSTRACT

Diabetes mellitus (DM) is a metabolic disorder characterized with hyper/hypoglycaemia, hypo/hyperinsulinemia. DM is associated with two broad metabolic complications viz; Short term complications including; diabetic ketoacidosis (DKA), hyperosmolar non ketotic coma (hyperosmolar hyperglycaemia state (HHS)) and hypoglycaemia, and the Long term systemic complications which are of two types; micro vascular complications such as such as diabetic nephropathy, diabetic retinopathy, diabetic microangiopathy, and diabetic neuropathy and the macro vascular complications include cardiovascular disease, heart attacks and stroke. The hyperglycaemia activates different signaling mechanisms such as an increased polyol pathway, advanced-glycation end products (AGEs) formation, activation of Protein Kinase C (PKC) and hexosamine pathway leading to over expression of reactive oxygen species and subsequent development of diabetic complications. Hyperinsulinaemia may grossly affect nutrient homeostasis viz; cell uptake of amino acids (all cells) and fatty acids, stimulation of glycolysis, inhibition of gluconeogenesis (liver), synthesis of glycogen (liver and muscle), triglyceride (liver and adipose) and protein (all cells) DNA synthesis, gene expression and growth promotion (mitogenic effects) (all cells), Inhibition of apoptosis (all cells) Stimulation of the N^+ / K^+ ATPase 'pump' (adipose and muscle). In conclusion DM as progressive chronic disease is closely related with complications such as diabetic nephropathy, diabetic foot, and diabetic retinopathy, cardiovascular disease. All these conditions must not be unconnected with altered metabolic status due to persistent abnormal glucose and insulin levels.

KEYWORDS: Diabetes Mellitus, Complications, Oxidative Stress, Polyol Pathway.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of carbohydrate in which glucose is under utilised leading persistent high level of blood glucose due to insufficient amount of insulin or factors that oppose the action of insulin.[1] It is estimated that, the number of people with DM Worldwide was about 425 million in 2017.[2] In Africa alone about 16 million people are diabetic.[2] In Nigeria about 1.7 million individuals have DM.[2] Incidence of DM is expected to rise, with the projection of 629 million worldwide and 41 million in Africa by 2045.[2] Genetic conditions and environmental factors (including overweight, obesity, pollution and life style), and their complex interaction can contribute to development of DM.[3] The disease is characterized by hyperglycaemia caused by impaired insulin secretion or peripheral insulin resistance.[4] This trigger counter reactions and activities in which body fats and proteins are mobilized to counter the effect of pseudohypoglycaemia (cells starving in the mist of plenty blood glucose). These result into polyphagia, polydipsia and polyuria, with attending nutrient lost and body wastages which are the hall mark for DM.[5] As a progressive chronic disease, it is closely associated with complications such as diabetic nephropathy, diabetic foot, and diabetic retinopathy; it is also one of the leading causes of cardiovascular disease.[4] This review focuses on overview of diabetes mellitus and its complications with emphasis on the current understandings of cellular/molecular mechanisms of vascular complications seen in DM.

Classifications of Diabetes Mellitus

Diabetes mellitus can be classified into the following general categories

1. Type1DM could be due to autoimmune or any other means of beta-cell destruction, leading to absolute insulin deficiency or reduced plasma insulin levels.[6]
2. Type 2 DM is associated with a progressive loss of beta-cell insulin secretion capacity and or peripheral insulin resistance.[6]

3. Gestational diabetes mellitus (GDM) may be associated with hormonal imbalance; it is normally diagnosed in second or third trimester in pregnant women with no clear overt DM prior to gestation.[6]

4. Other Specific types of DM e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation).[6]

Criteria for the diagnosis of diabetes mellitus

DM may be diagnosed when; Fasting Plasma Glucose is ≥126 mg/dL (7.1 mmol/L). Fasting is defined as abstaining from food intake for atleast 8 hours (usually overnight). Two (2)-hour Postprandial Glucose ≥ 200mg/dL (11.1mmol/L) during OGTT. The OGTT should be performed as described by the WHO, using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water. HbA1C≥6.5% (48 mmol/mol) is also used in diagnosis. In a patient with classic symptoms of hyperglycaemia such as polyphagia, polydipsia and polyuria or hyperglycaemic crisis, a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L) is diagnostic. In the absence of unequivocal hyperglycaemia, results should be confirmed by repeat testing.[2]

Complications of Diabetes Mellitus

Diabetic complications are of two types

I. Acute metabolic complications such as diabetic ketoacidosis (DKA), hyperosmolar non ketotic coma now referred to as hyperosmolar hyperglycaemic state (HHS) and hypoglycaemia

II. Long term systemic complications are of two types: Micro vascular complications such as such as diabetic nephropathy, diabetic retinopathy, diabetic microangiopathy, and diabetic neuropathy. Macro vascular complications include cardiovascular disease, heart attacks and stroke.[7]

Hyperglycaemia causes the activation of different signaling mechanisms such as an increased polyol pathway, advanced-glycation end products (AGEs) formation, activation of Protein Kinase C (PKC) and hexamine pathway which lead to over expression of reactive oxygen species and causes pathogenesis of diabetic complications. Glucose is the driving force in microvascular complications of diabetes mellitus. In type 2 diabetes mellitus, insulin resistance, and the metabolic syndrome, the vasculature is exposed to an assault by hypertension, dyslipidaemia, inflammation, and impaired fibrinolysis.[8] Chintan *et al.*[9] have characterized four major cellular signaling pathways that are activated by hyperglycaemia (figure 1) in endothelial cells and other cell types vulnerable to hyperglycemic attack. These include.

i. Activation of PKC (via diacylglycerol)
ii. Increased hexosamine pathway flux
iii. Increased advanced glycation end product (AGE) formation, and
iv. Increased polyol pathway flux.

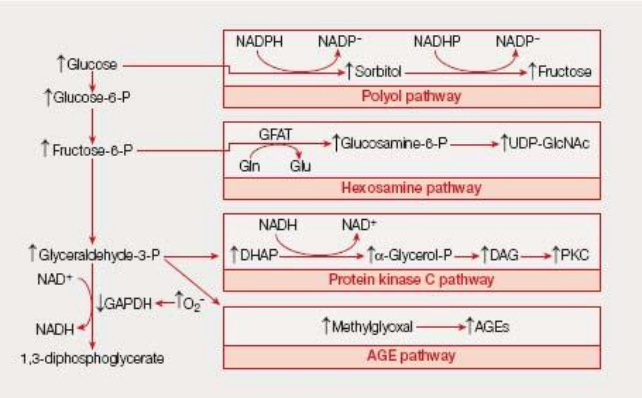

Figure. 1: Biochemical pathways leading to DM complications.

Chintan[9] proposed the existence of a unifying mechanism that integrates the above pathways: increased production of reactive oxygen species (ROS) (specifically superoxide) by the mitochondrial electron transport chain. Chintan[9] proposal may associates not only with hyperglycaemia, but also with hyper insulinaemia. The hormone that influence Na – ATPase pump in adipose tissue and muscle and glycolysis in liver which fuel proeiten kinase c pathway with concomitant insufficient reducing equivalents to oxidative phosphorylation. All these may culminate to ROS production.

Mechanisms of Vascular Complications In Diabetes

These involve interplay of a number of mechanisms in the pathogenesis of diabetic complications.

I. Advanced Glycation End Product (AGE)-RAGE Pathway

AGEs are compounds that have undergone posttranslational modifications irreversible as a result reactions between glucose and amino groups on proteins and nucleic acids. Hyperglycaemia as seen in DM accelerates the formation of AGEs, which accumulate in the extracellular matrix of vessels and contribute to vascular damage in diabetes mellitus.[7] AGEs stimulate production of reactive oxygen species (ROS) enhancing oxidative stress, which in turn aggravates AGE formation creating a vicious cycle. AGEs are also antigenic and can therefore induce immune responses leading to self attcak. In addition to AGEs, dicarbonyl methylglyoxal (figure 1), a by-product of glycolysis, via Amadori's reangements accumulates in tissues and contributes to diabetes-associated vascular damage.[7] AGEs interact with 2 main types of cell surface receptors.

(1) Scavenger receptors, which remove and degrade AGEs, and (2) Receptors for AGEs (RAGE), which trigger specific cellular signaling responses once AGE bind to it. RAGE is a member of the immunoglobulin family and binds many ligands besides AGEs, such as high mobility group protein B1, S100 calcium binding proteins (including calgranulin), amyloid- b-protein, and amphotericin. Petrie *et al.*,[10] reported that, AGE-RAGE transmits signals through transforming growth factor (TGF)-beta, NF- kB, mitogen-activated protein kinases, and nicotinamide adenine dinucleotide phosphate (NADPH) dependent oxidases (NOX) and induces expression of vascular adhesion molecule 1, E-selectin, vascular endothelial growth factor (VEGF), and proinflammatory cytokines (IL-1 b, IL-6, TNF- a). In DM there is increased activation of these signaling pathways in vascular smooth muscle cells due to increased AGE/RAGE production, leading to vascular fibrosis, calcification, inflammation, prothrombotic effects, and vascular damage. These increased activations of signaling pathways are very important processes leading to diabetic nephropathy, retinopathy, neuropathy, and atherosclerotic vascular diseases. Patients with diabetes mellitus have increased tissue alongside higher circulating AGEs and soluble RAGE concentrations, which can serve as predictive marker of cardiovascular events and all-cause mortality. As such, urinary and plasma AGE levels and soluble RAGE may act as biomarkers for vascular disease in diabetes mellitus.[10]

II. Activation of the Polyol Pathway

The polyol pathway originates from glycolytic pathway and consists of two main steps; the rate-limiting enzyme catalysed step where NADPH is used as a coenzyme by aldose reductase to reduce glucose to sorbitol. Then second step where sorbitol is oxidized to fructose via sorbitol dehydrogenase and NAD^+ is used a coenzyme (figure 2). Hyperglycemia is more likely to cause metabolic disorders via the activation of polyol pathway (figure 1) in tissues where glucose uptake is mediated by glucose transporters whose action are not insulin-dependent (e.g. glucose transporter 1) example eyes, nerves and kidneys. Glucose taken into the cell has a high affinity to glucokinase and undergoes extensive phosphorylation, but about 5% of glucose is not phosphorylated, but metabolized directly through the polyol pathway into sorbitol and fructose (Alan and Karin, 2009). When there is persistent hyperglycaemia, the percentage of glucose metabolized through the polyol pathway increases 4 to 5 times of normal making these tissues more susceptible to Diabetic complications.[11]

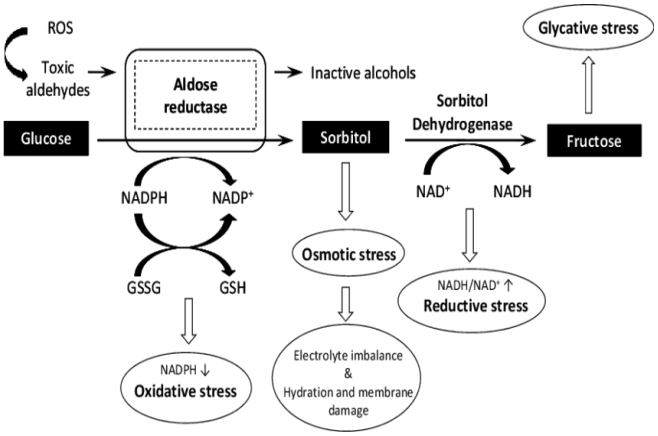

Figure. 2: Polyol Pathway leading to diabetic complications.

It is believed that the activation of the polyol pathway induces vascular damage directly and indirectly, for the following reasons

(1) Fructose and its metabolites, e.g., triose phosphate, methylglyoxal, fructose 3-phosphate, and 3-deoxyglucosone, are potent glycating agents. Accelerated production of these intermediates through the polyol pathway means more AGEs are to be produced.

(2) In the polyol pathway where NADPH is converted to $NADP^+$ and NAD^+ is converted to NADH, the depletion of NADPH decreases the production of reduced glutathione, which accelerates oxidative stress.

(3) An increase in NADH levels induces an increase in glycerol 3-phosphate levels, which activates protein kinase C (PKC) which provides another mechanism for development of diabetic complications.

In a study of diabetic ApoE knockout mice by Petrie *et al.*,[10], atherosclerosis was accelerated when the polyol pathway was activated via over expression of human aldose reductase and reduced when the polyol pathway was blocked by aldose reductase inhibitors.

III. Activation of Protein Kinase C (PKC)

PKC is a serine/threonine kinase activated by calcium and diacylglycerol (DAG). It plays an important role in intracellular signaling pathways. It is stimulated by different substances such as cytokines, growth factors, and vasoactive substances. PKC has many isoforms that are classified based on their structure and mechanism of activation: (I) Classical (or conventional) PKC (cPKC), (II) Novel PKC (nPKC), and (III) Atypical PKC (aPKC). In hyperglycaemic state, excessive amount of glucose is taken up into cells and glycolytic pathway is accelerated hence increased denovo synthesis of DAG from glucose. Since in the presence of high glucose levels, the polyol pathway and poly (ADP-ribose) polymerase are activated and the NADH/NAD＋ ratio increases, the NAD-dependent glycolysis from glyceraldehydes 3-phosphate (GAP), an aldose, to pyruvic acid is inhibited, while levels of dihydroxyacetone phosphate (DHAP) elevated, which results in an increased production of DAG.[12] DAG activates cPKC and nPKC. Experiments in diabetic animal models have demonstrated that an increase in

DAG levels in the heart, aorta, and renal glomeruli correlate with the activity of cPKC (α, β1, β2) and nPKC (δ, ε). Elevated oxidative stress associated with DM is also an important activator for PKC.[12]

PKC activation causes many abnormal changes related to atherosclerosis, such as an increase vascular permeability, activation of NADPH dependent oxidase, endothelial dysfunction, and impaired vasodilation due to decreased Nitric oxide production.[10]

IV. Enhanced Oxidative Stress

An increase in oxidative stress accelerates the progression of atherosclerosis and increases the risk of cardiovascular events by inducing inflammatory reactions, endothelial dysfunction, thrombogenic tendency, plaque instability, and the migration, proliferation, and transformation of smooth muscle cells. In patients with DM, oxidative stress is increased due to glucose auto-oxidation, enhanced activation of AGEs-RAGE axis, enhanced polyol pathway, increased influx into hexosamine pathway and increased PKCs activation, among others.[13] The production of ROS in mitochondria is increased in patients with DM (figure 3). Even in normal physiological situations, superoxides are generated as byproducts of oxidative phosphorylation in the mitochondrial electron transport chain, but when blood glucose levels are high, glycolysis is enhanced, which consequently increases the electrons flow to the mitochondrial electron transport chain and hence increased superoxides generation in the cells. It is believed that the activation of hexosamine pathway, polyol pathway and PKC is closely related to oxidative stress (figure 3) seen in diabetic patients.[13] As mitochondrial DNA is not protected by histones and is located near the inner mitochondrial membrane that contains the enzymes of the electron transfer system, it is susceptible to oxidative damage caused by ROS. Mitochondrial DNA injury decreases the production of ATP leading to cellular ATP depletion with subsequent tissue injury.[14]

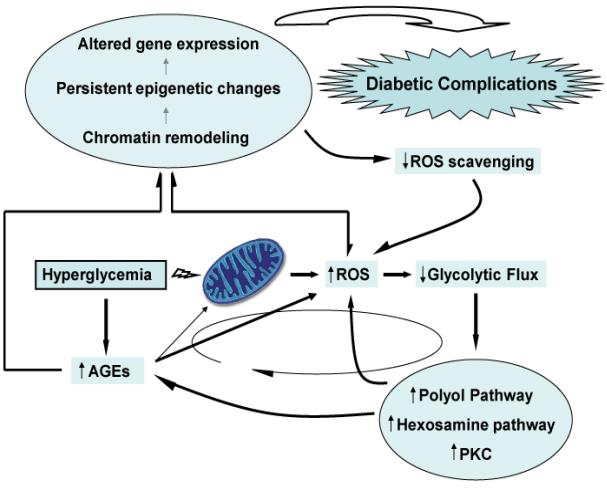

Figure. 3: Genetic, epigenetic and biochemical factors leading to diabetic complications.

It has been demonstrated that administration of a superoxide scavenger to diabetic mice led to a decrease in mitochondrial fragmentation through decreasing ROS.[15] ROS being free radicals react with components of the body, such as lipids, proteins, and nucleic acids to degenerate them. ROS induce erroneous expression of many genes through their direct effects or by promoting AGE production or activating PKCs, which leads to the onset and progression of complications (figure 3). Genes that are known to be affected by ROS include genes coding for Catalase and other anti-oxidant enzymes, Heme oxygenase-1, metallothionein-1 and other stress-response proteins, VEGF, monocyte chemoattractant protein-1, and other cellular growth factors and cytokines.[13]

V. Role of Endothelial Nitric oxide Synthase (eNOS) in Vascular Endothelial Dysfunction in DM

Major weapon of endothelial cells to fight vascular disease is eNOS. This enzyme generates vasoprotective molecule nitric oxide (NO) which is very important signaling molecule in vascular homeostasis. Tetrahydrobiopterin (BH_4) is identified as a critical cofactor in the activity of eNOS and its highly sensitive to oxidation. Diminished BH_4 bioavailability promotes eNOS uncoupling (figure 4) implicated in vascular complications in DM.[16]

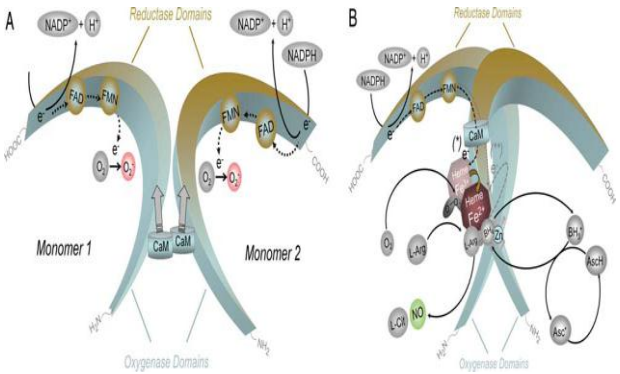

Figure. 4: Nitric oxide synthase structure (A= uncoupled NOS due to BH_4. B= NOS with normal activity).

The enzyme is constitutively expressed in endothelial cells and is localized to caveolae, which are specialized invaginations of the plasma membrane that are rich in specific lipids and proteins, including caveolin-1.[16] However, as the endothelial cells are exposed to risk factors such as cigarette smoke, high blood pressure, hyperglycaemia, or hyperlipidaemia as seen in DM, there is alteration of signaling pathways leading to eNOS uncoupling in the endothelium which is an important mechanism of endothelial dysfunction in diabetes mellitus.[16] These risk factors lead to excess production of superoxide (producing oxidative stress). Superoxide reacts with NO to form peroxynitrite (which is more destructive than superoxide), and vascular protection by NO slowly diminishes.[17] This reaction between superoxide and NO is only the beginning of the calamity

as eNOS enters into a vicious biochemical cycle; it changes its enzymology and starts producing peroxynitrite itself, and eventually becomes an enzyme that generates only superoxide.[18] This process of eNOS transformation to an enzyme that generates only superoxide is referred to as eNOS uncoupling. eNOS uncoupling plays a major role in endothelial dysfunction seen in diabetes mellitus.[16] eNOS has numerous cofactors of which tetrahydrobiopterin (BH_4) emerged as critical (figure 4). BH_4 is highly sensitive to oxidation by peroxynitrite and diminished level of BH_4 promotes eNOS uncoupling.[19] This transformation of eNOS from a protective enzyme to a contributor to oxidative stress has been observed in several in vitro studies and animal models of cardiovascular diseases, and in patients with cardiovascular risk factors such as diabetes mellitus.[19] In many cases, supplementation with BH_4 has been shown to correct eNOS dysfunction in animal models and patients.[20] NO produced by eNOS diffuses locally in the arterial wall and activates guanylyl cyclase in vascular smooth muscle cells, platelets, and endothelial cells to induce its biological effects. Abnormalities in this function contribute to atherosclerosis and its complications. NO bioavailability protects the blood vessels from atherosclerosis by mediating molecular signals that prevent platelet and leukocyte interaction with vascular beds.[16]

Genetic Link Between Type 2 Dm and Diabetic Complications

Loci Associated with Type 2 DM and its Complications

Many researchers[21][12] have addressed genetic polymorphism associated with diabetic complications (figure 5) and the list of loci and genetic variant linked to each specific complication is increasing, as more loci are being discovered. Up to now, at least 83 loci have been associated with type 2 DM and more than 30 with cardiovascular disease (CVD).[12] Figure 5 below summarised the variant of some genes that are associated or predisposes an individual to the development of diabetes mellitus and its complications and their link to biochemical pathways leading to the development of diabetic complications as discussed above.

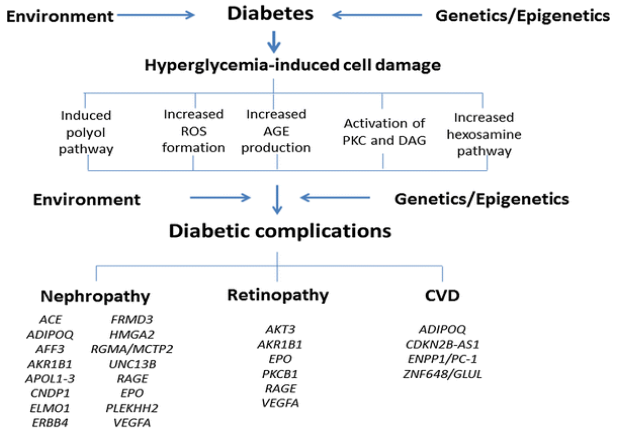

Fig. 5: Genetic polymorphism of Pathways leading to diabetic complications

Current findings on the genetic polymorphism with the genes variants implicated in predisposing individuals to diabetic complications are summarized in Table 1 below. Among candidate genes, the followings have been extensively investigated.

Table. 1: Genes whose variants are implicated in the development of both diabetes mellitus and its complications

Gene	Relative protein function	Risk of diabetic complications by genetic variant (s)
Adiponectin	Adipokine with anti inflammatory and antiatherogenic effects	↑ Risk
ADIPOR1	Adiponectin receptor. Metabolism of fatty acids and glucose	↑ Risk
ApoE	Lipoprotein transport	↑ Risk
CDKN2A/2B	Cyclin-dependent kinase inhibitor. Cell cycle regulation	↑ Risk
CELSR2-PSRC1-SORT1	CELSR2 is part of the cadherin superfamily, involved in contact-mediated communication. Proline- and serine-rich coiled-coil 1 plays an important role in mitosis. Sortilin 1 plays a role in the trafficking of different proteins to either cell surface or subcellular compartments	↓ Risk
GLUL	Enzyme implicated in ammonia and glutamate detoxification, acid–base homeostasis, cell signaling, and cell proliferation	↑ Risk
HMGA1	High-mobility group A1, architectural transcription factor with a role in cell growth, differentiation, and glucose metabolism	↑ Risk
HNF1A	Hepatic nuclear factor 1A, involved in development and metabolic homeostasis	↑ Risk
HP	Haptoglobin. Hemoglobin-binding capacity. Implicated in angiogenesis and in cholesterol-crystallization-promoting activity	↑ Risk
Paraoxonase	Enzyme that protects against lipid oxidation	↑ Risk
PCSK9	Proprotein convertase subtilisin/Kexin type 9. Plasma cholesterol metabolism	↓ Risk
PHACTR1	Phosphatase and actin regulator 1. PHACTR1 binds actin and plays a role in the reorganization of the actin cytoskeleton	↑ Risk

SOD2	Superoxide dismutase 2 transforms toxic superoxide into hydrogen peroxide and diatomic oxygen	↑ Risk
TCF7L2	Transcription factor 7-like 2, a member of the Wnt signaling pathway	↑ Risk

Epigenetic Changes

Epigenetic changes are defined as heritable modifications in gene expression that occur in the absence of changes in the sequence of DNA, it includes DNA methylation, histone acetylation, and RNA-based mechanisms (figure 6). These changes are cell specific and responsive to the environment, and should as far as possible be taken into consideration when investigating the hidden causes of diabetes mellitus and its complications.

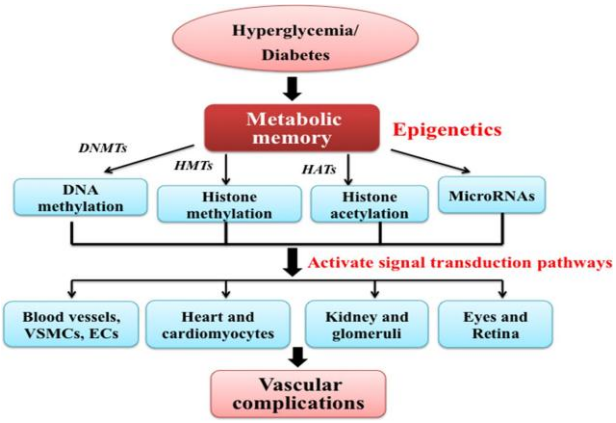

Figure. 6: Epigenetic factors linked to diabetic complications.

DNA or Histone Modifications

Persistent hyperglycaemia, induce epigenetic changes that lead to the overexpression of genes implicated in vascular inflammation. Hyperglycemia has been shown[12] to activate the NF-Kb signaling pathway in cultured THP-1 monocytes, leading to the production of MCP-1 and other inflammatory factors, and to the expression of adhesion molecules in endothelial cells, providing a plausible molecular mechanism for endothelial dysfunction and atherosclerosis. In support of an epigenetic role of hyperglycemia, DeRosa *et al.*,[12] demonstrated that exposure to high glucose in aortic endothelial cells, correlates with the inverse acetylation of the histone H3K9/K14 and modified DNA methylation patterns. The interplay of many other biochemical mechanisms in diabetes mellitus, independent of glucotoxicity, like ROS, PKC activation, and AGEs which have been discuss earlier (figure 3), are known to induce epigenetic changes.[17] An association between IGF2 methylation and lipid profile alterations was found in obese children. In particular, IGF2 hypermethylation was associated with higher triglyceride/HDL-cholesterol ratio, representing an epigenetic marker of metabolic risk.

Abnormalities in MicroRNA (miRNA) Expression

MicroRNAs are small single-strand RNA molecules that influence their target genes at a posttranscriptional level, thereby regulating many biological processes.[12] Since their discovery about two decades ago, numerous miRNAs have been described to be associated with many diseases, including type 2 DM and CVD. In particular, with reference to type 2 DM, miRNAs have shown to be involved in regulating beta cell function, insulin response, glucose homeostasis, as well as the pathogenesis of diabetic vascular complications (Tang *et al.*, 2017). It has been demonstrated that, in the presence of high glucose concentrations, up regulation of miR-185 reduced the expression of the glutathione peroxidase-1 (GPx-1) gene, which encodes an enzyme important in the prevention of oxidative stress. In the endothelium, miR-126 exerts proangiogenic, and anti-inflammatory activities. At a functional level, it enhances VEGF and fibroblast growth factor activities, contributing to vascular integrity and angiogenesis, recruits progenitor cells, while it suppresses inflammation by inhibiting TNF-α, ROS, and NADPH oxidase.[23]

CONCLUSION

Diabetes mellitus, a progressive chronic disease, is closely related with complications such as diabetic nephropathy, diabetic foot, diabetic retinopathy and a leading causes of cardiovascular disease. CVD in turn is the leading cause of mortality in diabetic patients. Biochemical mechanisms involved in the development of diabetes mellitus complications includes; an increased polyol pathway, advanced-glycation end products (AGEs) formation, activation of Protein Kinase C (PKC) and hexosamine pathway lead to the over expression of reactive oxygen species leading to the development of diabetic complications. These pathways are also linked to genetic and epigenetic factors responsible for the development and progression of complications seen in diabetes mellitus.

Table of Abbreviations

AGEs	Advanced-glycation end products
BH_4	Tetrahydrobiopterin
CVD	cardiovascular disease
DAG	Diacylglycerol
DHAP	Dihydroxyacetone phosphate
DKA	Diabetic ketoacidosis
DM	Diabetes mellitus
eNOS	Endothelial Nitric oxide Synthase
GAP	Glyceraldehydes 3-phosphate
HbA1C	Glycated Haemoglobin
HHS	Hyperosmolar hyperglycaemia state
IGF	Insulin-like growth factor
IL	Interleukin
MODY	Maturity-onset diabetes of the young
NADPH	Nicotinamide adenine dinucleotide phosphate
NF	Nuclear factor
NO	Nitric oxide
NOX	NADPH-dependent oxidases
OGTT	Oral glucose tolerance test

PKC	Protein Kinase C
RAGE	Receptor for advanced-glycation end products
ROS	Reactive oxygen species
TGF	Transforming growth factor
VEGF	Vascular endothelial growth factor

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