Molecular insights into diabetic cardiomyopathy

Chayanika Barman*, Rajesh Pandey, Jasbir Singh, Kuldip S. Sodhi

Department of Biochemistry, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Ambala, Haryana, India

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*Correspondence:
Chayanika Barman,
E-mail: Chayanikabaran123@gmail.com

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ABSTRACT

Diabetes mellitus affects the heart in 3 ways: (1) coronary artery disease (CAD) due to accelerated atherosclerosis; (2) cardiac autonomic neuropathy (CAN); and (3) diabetic cardiomyopathy (DbCM). Although there is high awareness among clinicians about the first two entities, DbCM is poorly recognized by most physicians and diabetologists. DbCM, first described by Rubler et al. in 1972, is defined as myocardial dysfunction occurring in patients with diabetes in the absence of CAD, hypertension, or valvular heart disease. The development of DbCM is multi-factorial including autonomic dysfunction, metabolic derangements, abnormalities in ion homeostasis, alteration in structural proteins, and interstitial fibrosis. Chronic hyperglycemia is thought to play a central role in the development of DbCM. The main metabolic abnormalities in diabetes are hyperglycemia, hyperlipidemia and inflammation, all of which stimulate generation of reactive oxygen/nitrogen species which result in reduction of myocardial contractility and acceleration of fibrosis besides cellular DNA damage and cardiomyocyte apoptosis. In addition, advanced glycation end products (AGEs) indirectly exert their detrimental effect on the myocardium by interacting and up-regulating their receptors, including receptors of AGE and galectin-3. This results in activation of transcription factors, such as nuclear factor-kB (NF-kB). The NF-kB dependent genes, in turn, trigger several pathways that induce production of pro-inflammatory cytokines and cause myocardial damage. All these molecular events are potential therapeutic targets in DbCM.

Keywords: Diabetic cardiomyopathy, Insulin, Genes, Apoptosis, Fibrosis, Therapy

INTRODUCTION

Diabetes mellitus (DM) may be considered as one of the challenges even in the highly developed medical field of 21st century. It is becoming an epidemic health threat.1 It affects 350 million people around the world, and the WHO has projected that diabetes deaths will be double between 2005 and 2030.2 It is a syndrome characterized by hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and, or insulin action.3 The disease affects almost every tissue in the body and causes significant organ dysfunction with acute and long term complications that result in diabetes related morbidity and mortality.4,5

Heart disease has been singled out as a major cause of death in patients with DM as diabetes increases the risk of developing heart disease by several fold.5,6 Heart involvement in diabetes goes beyond the damage to coronary arteries due to the progress of atherosclerotic process. Diabetes and its pathophysiological consequences are able to induce direct alterations and abnormalities in the cardiac muscle functions.5 Several studies have suggested that diabetes may be associated with left ventricular (LV) structural and functional abnormalities in addition to, and independent of atherosclerosis.3 Diabetes may affect heart in three ways: (1) coronary artery disease (CAD) due to accelerated atherosclerosis; (2) cardiac autonomic neuropathy (CAN); and (3) diabetic cardiomyopathy (DbCM).3
Although there is high awareness among clinicians about the first two disease entities, DbCM is poorly recognized by most physicians and diabetologists.8

It is worth to be mentioned that there is increasing evidence of diabetics with abnormalities of left ventricular function in the absence of clinical heart disease and this is what called as diabetic cardiomyopathy.9 The high morbidity and mortality for DbCM warrant aggressive clinical management.1 In addition, the increasing detection of this added cardiac insult is supported by data from epidemiological, molecular as well as diagnostic studies.6

This review is aimed to get a general perspective mainly on the molecular insights in the pathogenesis and in addition to that the early diagnosis and management of DbCM which may support the improvement of concern regarding DbCM.

Definitions

Most cardiac muscle diseases are secondary to some other condition, e.g., coronary atherosclerosis, hypertension or valvular heart disease. However there are also cardiac diseases attributed to intrinsic myocardial dysfunction. Such diseases are termed as cardiomyopathies (literally, “heart muscle diseases”).7 DbCM is the presence of myocardial dysfunction in patients with diabetes in the absence of other known cardiac disease.8 This entity was originally described by Rubler et al in 1972 on the basis of observations on four diabetic patients who presented with heart failure without evidence of hypertension, CAD, valvular or congenital heart disease. Since then, DbCM has been defined as ventricular dysfunction (systolic and diastolic) that occurs independently of CAD and hypertension.6 It is a disease process which affects the myocardium in diabetic patients causing a wide range of structural abnormalities eventually leading to left ventricular hypertrophy (LVH) and diastolic and systolic dysfunction or a combination of these. The concept of DbCM is based upon the idea that diabetes is the factor which leads to the changes at the cellular level, leading to structural abnormalities.9

Epidemiology

In diabetic patients, the prevalence of DbCM is 12% and reaches 22% in people over 64 years old.10 The Framingham study was the first to demonstrate an increased risk of heart failure in patients with DM.3 It showed that the frequency of coronary artery disease is twice more common in diabetes patients of both sexes.11 The prevalence of different degrees of heart failure among diabetic subjects was as high as 19%-26% in different major clinical trials.4 The risk of CAD increases rapidly after the age of 40 in patients with type1 diabetes, and by the age of 55, 35% of the patients of both sexes have died because of coronary artery event, compared with 8% of men and 4% of women in general population.

The increased percentage of patients with diabetes and heart failure in numerous multicenter epidemiological studies is in contrast to the total diabetic burden in the general population (6%-8%). In the Studies of Left Ventricular Dysfunction (SOLVD), the percentage of patients with diabetes and heart failure was up to 26%, whereas in the Assessment Trial of Lisinopril and Survival (ATLAS) study, it was 19% and in the Vasodilator-Heart Failure Trial II (V-HeFT II), it was up to 20%. These findings are in agreement for the presence of an additional detrimental factor in the diabetic myocardium, which predisposes it to extensive damage followed by heart failure.11

Molecular Insights (Pathogenesis)

The pathogenesis of DbCM is multifactorial including autonomic dysfunction, metabolic derangements, abnormalities in ion homeostasis, alteration in structural proteins, and interstitial fibrosis.12 Hyperglycaemia, hyperlipidaemia and increased reactive oxygen species (ROS) induce alterations in downstream transcription factors which result in changes in gene expression, myocardial substrate utilization, myocyte growth, endothelial function and myocardial compliance.9 Some major molecular abnormalities and their involvement in DbCM are discussed below (Fig. 1):-

Figure 1: Molecular aspects of DbCM.

A. Hyperglycaemia and DbCM

Chronic hyperglycemia results in a number of metabolic and molecular changes in the myocardial cells. Increased glucose metabolism due to hyperglycaemia leads to an increase in oxidative stress by generation of ROS from mitochondria. Overproduction of superoxide by the mitochondrial respiratory chain and the consequent oxidative stress result in reduction of myocardial contractility and eventually myocyte fibrosis. ROS and oxidative stress can cause cellular DNA damage and acceleration of cardiomyocyte apoptosis. DNA damage induced by oxidative stress also activates poly ADP ribose polymerase (PARP), a DNA reparative enzyme. PARP diverts glucose metabolism from its usual glycolytic pathway (through inhibition of glyceraldehyde
phosphate dehydrogenase) into alternative biochemical pathways that result in generation of various mediators which causes hyperglycemia induced cellular injury. These include advanced glycation end products (AGEs), increased flux of hexosamine and polyol, and activation of the enzyme protein kinase C. AGEs can covalently crosslink various intra and extracellular proteins that is thought to be a pivotal factor in diabetic complications. The crosslink in collagen and elastin results in increased myocardial stiffness and impaired cardiac relaxation. AGEs are found to induce myocardial damage in human beings. AGEs also indirectly exert their detrimental effect on the myocardium by interacting and up-regulating their receptors, including receptors of AGE and galectin-3. This results in activation of transcription factors, such as nuclear factor-kB (NF-kB). NF-kB dependent genes in turn trigger several pathways that induce production of pro-inflammatory cytokines such as Tumour necrosis factor-a and cause myocardial damage. PKC (protein kinase C) activation by hyperglycemia contributes to cardiac fibrosis by stimulating connective tissue growth factor (CTGF) expression as shown in transgenic PKC-b2 mice. Consistently, the overexpression of PKC-b2 isoform in the heart of transgenic mice resulted in LV hypertrophy, fibrosis and decreased LV ejection fraction (LV EF), similar to that of DCM. PKC phosphorylates and subsequently activates IkB kinase. IkB kinase phosphorylates serine residues on insulin receptor substrate-1 (IRS-1), inhibiting its ability to bind SH2 domains of the p85 regulatory subunit of phosphatidylinositol 3-kinase (PI3K), impairing insulin signal transduction. Hyperglycemia activates intra cardiac renin-angiotensin system (RAS) that has various effects on the myocardial cells. An increased expression of angiotensin-II (AGT-II) in diabetic heart rate has been related to cardiomyocyte hypertrophy and apoptosis. AGT-II has a direct effect on cell signaling that results in hypertrophy in cardiac myocytes and proliferation of cardiac fibroblasts. Other factors, such as oxidative stress, inflammation and aldosterone, may contribute to deleterious effects of AGT-II on the heart producing myocardial damage in diabetes. All these factors ultimately lead to DbCM.

B. Hyperlipidemia and DbCM

In diabetes, there occurs elevation of circulating free fatty acids (FFA) because of enhanced adipose tissue lipolysis and hydrolysis of augmented myocardial triglyceride stores. High circulating as well as cellular levels of FFA results in abnormally high oxygen requirements during FFA metabolism and intracellular accumulation of potentially toxic intermediates of FFA like ceramide, all of which can lead to impaired myocardial performance, severe morphological changes and particularly it is potent in inducing apoptosis in cardiomyocytes. The lipotoxicity due to toxic metabolites from FFA opens K-ATP channels and this impairs the ability of cardiomyocytes to regulate calcium use, causing contractile dysfunction. The FFA induced impairment of glucose oxidation is a major factor in the development of diabetic cardiomyopathy. In the heart of diabetics, energy production by glucose utilization may be decreased and FFA utilization is increased and this causes depletion of glucose transporter (GLUT)-1 and -4. Nonesterified fatty acids (NEFAs) play a critical role in triggering the development of cellular insulin resistance but also have been implicated in the development of myocardial contractile dysfunction. They play a central role in altering cellular insulin signaling through several mechanisms leading to insulin resistance and compensatory hyperinsulinemia. In turn, hyperinsulinemia is an important trigger to the development of cardiac hypertrophy in diabetic cardiomyopathy. NEFAs also directly alter myocardial contractility independent of altered insulin action by increasing NEFA flux into the myocardium. Increased FA is also associated with the activation of peroxisome proliferator-activated receptor-α (PPAR-α), and its activation induces pyruvate dehydrogenase kinase-4 causing glucose oxidation and stimulating fatty acid uptake in the mitochondria. Along with an increase in long chain acyl carnitines, it promotes mitochondrial uncoupling of oxidative phosphorylation which in turn results in decreased myocardial high energy reserves and contractile dysfunction.

C. Inflammation and DbCM

Inflammatory signaling in cardiomyocytes usually occurs as an early response to myocardial injury and entails cytosolic and mainly mitochondrial ROS overproduction. Toll like receptors (TLRs) are membrane anchored proteins present in several cell types ranging from macrophages and T and B cell to nonimmune cells like cardiomyocytes. They work as pattern recognition receptors (PRRs) implicated on tailoring innate immune signaling. TLRs elicit conserved inflammatory pathways culminating in the activation of NFkB and activating protein-1 (AP-1). TLR ligands include high mobility group B1 (HMGB1), heat shock proteins (HSP60, HSP70), endotoxins and extracellular matrix components. Also ROS can modify membrane components and cause the release of the factors that intact with and active TLRs. Several studies have addressed the role of TLRs in cardiac inflammation using models of T1DM, T2DM and obesity, which share an environment characterized by high circulating levels of glucose and FFA with TLRs has been described, high levels of glucose and lipids have been shown to stimulate TLR2 and TLR4, thus suggesting the existence of unknown intermediates. High fat diet induced obese mice exhibited myocardial macrophage infiltration as well as higher expression levels of TLR4, MyD88 and IL-6. Consistence with this, both diabetic TLR2 and TLR4 deficient mouse hearts showed lower triglyceride accumulation during the early stage of diabetes, as well as restricted leucocyte infiltration and a marked decrease of NFkB and MyD88 and phosphorylation of IRAK1. The inflammasome is a group of multimeric protein complexes composed of a
cytoplasmic receptor of the Nod-like receptor (NLR) family, an adaptor protein termed ASC (Apoptosis-associated speck-like protein containing an N-terminal caspase recruitment domain CARD) and procaspase-1. The best characterized complex is the NLRP3 inflammasome, which has been identified in a wide range of cells including macrophages, cardiofibroblasts, and cardiomyocytes. NLRP3 has been reported to be held in an inactive state by cytoplasmic chaperones. Once NLRP3 is freed, subsequent oligomerization leads to the recruitment of procaspase-1, thus promoting autocleavage and activation. Active caspase-1 can eventually process IL-1β and IL-18 precursors, serving as enhancer of multiple proinflammatory pathways including NF-κB, mitogen-activated protein kinase (MAPK), IFNγ, chemokines, and ROS and also promoting insulin resistance. NLRP3 inflammasome may also participate in the cardiomyocyte and monocyte response in DbCM-associated inflammation. Activation of both PPARs and Sirt1 may control the TLR and inflammasome-dependent pathways of inflammation in DCM, which may be useful for a therapeutic target.18

D. Oxidative stress, transcription factor and DbCM

Cells contain a number of genes coding many proteins to counteract ROS, reactive nitrogen species (RNS), or electrophile-mediated injury. Transcriptional regulation of these protective genes is controlled in part through antioxidant response elements (AREs). The transcription factor nuclear factor NF-E2-related factor 2 (Nrf2) plays an important role in ARE-mediated basal and inducible expression of more than 200 genes that can be grouped into several categories including antioxidant genes and phase II detoxifying enzymes. Down-regulation of Nrf2 is a significant reason for the initiation of various diabetic complications. The effective protection from diabetic complications by up-regulating Nrf2 function in animal models promoted clinical trials with Nrf2 inducer to prevent diabetic nephropathy. In 2011, the phase II clinical trial by following-up 52 weeks for the treatment of participants with moderate-to-severe diabetic kidney disease with bardoxolone methyl (BM) reported the improvement of renal function compared to non-BM-treated diabetic patients. But the phase III clinical trial for the patients with advanced diabetic kidney disease was prematurely terminated due to the strong adverse effects associated with BM treatment, including increased rates of heart failure and cardiovascular events. The failure of BM clinical trial suggests that more detail study in preclinical animal models is urgently needed before new clinical trials. Given the efficient prevention of diabetic complications with Nrf2 inducers in various animal models and the escalating human and societal costs of diabetic complications, efforts to find new safe and effective drugs via up-regulating Nrf2 remain vital.

The detrimental effects of Nrf2 due to its aberrant activation have also been highlighted in recent years. A few of such examples include: (1) constitutive Nrf2 activation worsens insulin resistance, impairs lipid accumulation in adipose tissue, and increases hepatic steatosis in lepin-deficient mice; (2) Nrf2 deficiency improves glucose tolerance in mice fed a high-fat diet and (3) Nrf2 deficiency prevents reductive stress-induced hypertrophic cardiomyopathy.19 Given that oxidative and/or nitrosative stress seems a critical cause for the development of DbCM, several anti-oxidant approaches have been investigated for their potential prevention and amelioration of DbCM. Although anti-oxidant therapy has been extensively studied for its potential prevention or treatment of various diabetic complications, direct investigations for the prevention by anti-oxidants of DbCM remain relatively scarce. In recent studies, intramyocardial thioredoxin 1 (Trx1) gene therapy in diabetic rats showed the prevention of angiogenic impairment and cardiac dysfunction severity after cardiac infarction. They found that overexpression of the Trx1 gene could reduce oxidative stress and apoptosis and, thereby, the extent of ventricular remodeling. Super oxide dismutase (SOD) has been found to protect cardiomyocytes from superoxide-induced damage by converting superoxide to hydrogen peroxide. Tempol, 4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl, is a membrane permeable SOD mimetic that has been shown to attenuate the effects of peroxynitrite in vascular systems. In the diabetic heart, tempol was also found to significantly reduce diabetes-activated GSK-3β, associated with increases in caspase-3 cleavage and apoptotic cell death at 3 days after streptozotocin (STZ) injection. Curcumin is the natural polyphenolic compound that was originally used in traditional Indian medicine over 3,000 years ago, and has shown diverse pharmacological properties including anti-oxidant activity. Soetikno et al. have shown the prevention of DbCM by curcumin in a STZ-induced diabetic rat model, and also found that the prevention of DbCM by curcumin is associated with the suppression of NOX activation leading to the overgeneration of ROS and/or RNS, either through inhibition of PKC or activation of Akt pathways. Coenzyme Q10, a lipophilic cofactor of the mammalian mitochondrial electron transport chain, is not only crucial for mitochondrial energy production (adenosine 5′-triphosphate [ATP]), but also has emerged as an effective anti-oxidant. Coenzyme Q10 has shown some clinical benefits in a number of clinical trials. Resveratrol (RSV; trans-3,5,4′-trihydroxystilbene), a polyphenolic compound and naturally-occurring phytoalexin present in red wine and vegetable foods, was also used for the potential prevention or therapy for DbCM. In experimental models of diabetes, RSV treatment was found to reduce the generation of ROS and the incidence of cardiomyocyte death, along with improvement of cardiac function. Metallothionein (MT) is a cysteine-containing (1/3 of 61 amino acids) and metal-binding protein. Physiological functions of MT include the maintenance of the homeostasis of essential metals and detoxification of heavy metals. It maintains the redox status by binding and releasing metals (mainly zinc [Zn] under physiological conditions). MT-I and MT-II exist as
the major isoforms in various human and animal organs including the heart. MT has shown strong cardioprotection from diabetes under different conditions: type 1 diabetes (STZ-induced and OVE26 transgenic diabetic mouse models), obesity-related diabetes in mice and even in humans with type 2 diabetes. As Zn is an important trace element that is required for more than 300 enzymes and transcription factors, and can induce cardiac MT and MT plays an important role in intracellular Zn, Zn supplementation could be an alternative approach to intervention of DbCM in clinics in the future.13

**Diagnosis**

The clinical diagnosis of diabetic cardiomyopathy has two important components:6

a) Detection of myocardial abnormalities
b) Exclusion of other contributory causes of cardiomyopathy

The diagnosis of diabetic cardiomyopathy currently rests on non-invasive imaging techniques like echocardiography (ECG) and magnetic resonance image (MRI).6 The findings obtained by these methods are as follows:7–17

i) **Echocardiography**: In the early stage of DbCM and in the majority (75%) of asymptomatic diabetic patients, diastolic dysfunction characterized with heart failure with normal ejection fraction is present. Diastolic dysfunction, mitral inflow patterns, cardiac stiffness, and dilatation of the LV can be assessed by echocardiography. But these changes are not confined to DCM but are also present in other cardiac diseases and therefore, other approaches are required for diagnosing DbCM.

ii) **MRI**: MRI is a highly sensitive tool for detecting LV wall motion abnormalities, geometry, and cardiac hypertrophy. Additionally, it provides information on arrhythmia and cardiomyopathy.

Changes in the levels of various plasma/serum cardiac biomarkers may reflect some of the myocardial metabolic and structural functions.4 Some of them are:

- **Matrix metalloproteinases (MMPs)**: These are the enzymes that degrade extracellular matrix, increase metabolic turnover and alter the expression of several micro ribonucleic acids (mi-RNAs) that lead to contractile dysfunction of the myocardium. Elevated levels of MMPs especially MMP9 are seen in myocardial fibrosis.4
- **Serum aminopeptidase terminal of type III (PIIINP)**: An indicator of type III collagen turnover in the body, was suggested to be an early indicator of LV dysfunction in obese with insulin resistance.4
- **Cardiac troponin**: Present in the plasma, is an indicator of necrosis.17
- **Beta o-linked N-acetylglucosamine (β-O-GlcNAc)**: Levels of this enzyme can also be used as a predictor of DbCM as it is increased in hypertrophy and cardiovascular diseases.17
- **miRNA**: These are small (~22 nucleotide), conserved, non-coding RNA molecules which may serve as a potential biomarker of DbCM.17 Recent studies have also linked dysregulation of specific miRNA-1, which accounts for approximately 40% of the total myocardial miRNA pool, has been shown to downregulate Pim-1 in STZ-induced type 1 diabetic mice, and restoration of Pim-1 prevented cardiomyocyte apoptosis, ventricular dilatation and failure. Myocardial expression of miRNA -133 is increased in the alloxan-induced rabbit model of type 1 diabetes, and miRNA-133 modulates connective tissue content by regulating CTGF expression, suggesting its contribution to fibrosis induction in diabetic hearts.20

**Management**

- **Life style**: Improvement of an unhealthy life style is highly useful for prevention as well as control of diabetes mellitus and thereby decreases the risk of development of DbCM. Regular physical exercise, limitation of fat and total energy intake, stopping the habit of cigarette smoking etc. are significant.11
- **Glycemic control**: This is an important treatment aim in the management of DbCM.11 Diabetes control may be the most basic and important strategy for preventing the development of DbCM.12 Drug like metformin improves peripheral sensitivity to insulin, promotes hyperglycemic control, and acts as an anti-inflammatory agent. Glucagon-like peptide (GLP)-1 is an incretin hormone that stimulates postprandial insulin secretion and improves insulin sensitivity. Individuals treated with GLP-1 also have improved left ventricular ejection fraction. Recent meta-analyses suggest that the use of the beta-blockers improves glycemic levels and insulin resistance when compared with other antidiabetic drugs.17
- **Lipid lowering therapy**: There are no clinical trials investigating the role of lipid lowering therapy in human subjects with established DbCM, beneficial effects of the treatment of dyslipidemias can be anticipated in these patients along with a role in the primary prevention of the disease.4 Statin treatment has shown to reduce cardiovascular events and mortality in patients with diabetes and vascular risk factors in multiple clinical trials. Atorvastatin, independent of its LDL cholesterol lowering capacity, has shown to reduce intramyocardial inflammation and myocardial fibrosis, and improve LV function in rat models of experimental DbCM.4
- **Management of coexistent hypertension, CAD and heart failure**: There are no formal guidelines on the management of coexistent hypertension and
cardiac ischemia in patients with DbCM. However, when these diseases coexist, they accelerate the progression of DbCM because of their detrimental effects on ventricular function and structure. Optimal treatment of hypertension and CAD would be expected to ameliorate the disease progression and even slow it down.4
- **Trimetazidine**: It is an atypical anti-anginal agent (it inhibit β-oxidation of fatty acids) that shifts cardiac energy metabolism from free fatty acid oxidation to glucose oxidation. The drug has shown promising beneficial effect on heart failure in diabetic patients with both ischemic and idiopathic dilated cardiomyopathy. Animal model has shown that trimetazidine improved myocardial function by attenuating lipotoxicity and augmented oxidation status of the heart and might suppress the development of DbCM. Human trials are needed to investigate the beneficial effects of this well-tolerated drug on treatment and prevention of DbCM.4
- **Inflammasome targeting**: A new set of potential therapeutic approaches for DbCM may include the stimulation of PPARs and Sirt1 and the inhibition of TLR2, TLR4, and NLRP3. Further, targeting proximal TLR mediators MyD88 and IRAK and the activation steps of the inflammasome may yield some clinical benefit in DbCM.18

**CONCLUSION**

The key factors implicated in DbCM are enumerated in Table 1. Majority of these have been touched in this review. Several of the factors and their participation in signaling events offer potential therapeutic targets, and this should be the mainstay of future research in DbCM.

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