Review Article:

Oxidative stress causes cardio myocyte apoptosis: May be the determinant of development of myocardial disarray in diabetes

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Abstract:
Oxidative stress resulting from enhanced free radical formation or defect in anti-oxidant defence mechanism, implicates in the development of various disorders including impairment of vasodilatation, neuro degeneration, ventricular dysfunction and changes in cardiac muscle cells morphology. Oxidative stress activates renin aldosterone angiotensin system and inflammatory cytokines that induce apoptosis in heart muscles. It also decreases ATP formation that leads to apoptosis in myocyte. As a consequence, the physical force in the heart is increased that may guide to programme cardiac muscle cell death. The net effect of oxidative stress leads to an architectural rearrangement of the myocardium involving side to side slippage called myocyte disarray. Single myocyte cell death allows side by side translocation of cells. However, multiple cells death causes sliding of the myocyte bundle. No work has yet been done to correlate the myocardial cell slippage and oxidative stress in diabetes, in which heart muscles apoptosis are evident. Thus the present review is done to scrutinize if there is any role of oxidative stress on development of myocyte disarray in diabetes.

Key words: Oxidative stress, cardio myocyte apoptosis, myocardial disarray, diabetes

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Introduction:
Heart disease is the major cause of death inpatient, with diabetes (1). Risk of death is increased many fold if diabetes is associated with coronary artery disease (CAD) (2-4). In 1972, Ruber et al first proposed that diabetes independently impaired cardiac performance & caused overt heart failure without CAD, hypertension and congenital heart disease, on observation of four patients who died from heart failure (5). A number of experimental studies have been carried out to determine that what structural alteration deteriorates the cardiac functions in drug induced diabetic animals, but the results from these studies varied (6,7) Most have concentrated mainly on the ultrastructure of the cardiac myocyte, accumulation of fat, myofibril and collagen fibre (6-8). No study has yet been undertaken to correlate between diabetes and myocyte disarray. Literature shows that myocytes apoptosis may cause architectural rearrangement of the myocardium involving side-to-side slippage of myocytes (9-13). Heart muscle apoptosis is obvious in diabetes (14-17). Thus, there may be a possibility to develop myocardial disarray in diabetes that may lead to cardiac dysfunction.

Myocyte Disarray
Myocyte disarray was first reported by Teara in 1958 (18). It is defined as an area of myocardium where adjacent myocardial cells are aligned perpendicularly or obliquely to each other rather than its normal parallel alignment (19-21) Figure 1. It is usually found abundantly in the interventricular septum (20). It is also observed in the interventricular free wall (20, 21). It is a common structural lesion observed in many
cardiac diseases, including coronary heart disease, cor pulmonale, congenital heart disease (22-24) and hypertrophic cardiomyopathy (HCM) with (22,23,25) or without obstruction (25,26). Males are more predisposed to disarray than females (27). Disarray has no relation with heart weight (19, 28).

![Normal heart muscle](A) ![Myocardial disarray](B)

**Figure 1 Photomicrographs showing the (A) normal and (B) myocardial disarrayed**

Maron et al proposed that abnormally arranged cardiac muscle cells in HCM probably do not produce an efficient pattern of contractility and may be responsible for marked functional limitation and may causes premature sudden death (20). They found extensive disorganization in left ventricular wall at the necropsy of heart of a 25 years old patient in HCM, who died suddenly (20). Similar results have been found in 8 out of 9 young patients who died suddenly from HCM. Their heart had greater myocardial disarray (29). Animal studies also showed that 4 of the 7 cats who died suddenly and unexpectedly, had marked cardiac muscle cells disorganization in ventricular septum along with HCM (30). This disorganized myocardial cells arrangement may be found in diabetes and may cause morbidity and mortality, without CAD.

**Oxidative Stress and Myocyte Apoptosis**

In diabetic cardiomyopathy, hyperglycaemia causes significant reduction and cellular depletion of the glucose transporters GLUTs 1 and 4 (15). Due to unavailability of glucose as energy the intercellular metabolism is shifted from glycolysis to free fatty acid oxidation, resulting in increased production of free radical /reactive oxygen spices (ROS). The ROS elicits, an array of damage to the cells of heart including membrane lipid peroxidation, cross-linking, degradation of protein and nicking of DNA, ensuing the impairment of cellular integrity and function as well as apoptosis (15, 17). Moreover, pyruvate oxidation is also reduced by PPAR (peroxisome proliferation activated receptor), in diabetes. Thus the net result is an excessive glycolytic intermediate formation and an increased synthesis of ceramide leads to myocyte apoptosis (4).

On the other hand, free radicals are also generated from reduced sugar (Amadori products). Glycation and oxidation reactions between reduced sugars and proteins as well as lipids contribute to glycated end products or glycoxidation products (31). Advanced glycated end products (AGE) deactivate nitrous oxide (NO) and impair coronary vasodilatation. Accumulation of AGE modified extracellular matrix results in inelasticity of blood vessel wall that could interfere with myocardial function (32).

Sustained hyperglycaemia causes formation of mitochondrial ROS with effects on transcription of DNA that regulates the deposition of collagen in the myocardium and leads to contractile dysfunction (4).

In diabetic animals, AGE impairs SERCA 2a (Sarcoplasmic/endoplasmic reticulum Ca2+-ATPase 2a), which is responsible for depletion of intracellular Ca+ levels that play a role in cardiac relaxation (33, 34). Furthermore, oxidative damage depletes Na+ K+ ATPase and
enhances Na+/Ca++ transporters in cardiomyocytes and leads to an overload of intracellular Ca++; after 12 weeks in streptozotocin induced diabetic rats. This may be involved in impaired ventricular relaxation 1. Interstitial accumulation of AGE also has a greater effect on early diastolic filling compared to the late diastole, in diabetes alone, which indicates the primary dysfunction in the ventricle without CAD (35).

Insulin deficiency or resistance, independent of hyperglycaemia also causes cardiac apoptosis (36). Removal of insulin causes a significant increase in ROS production that result in oxidative mitochondrial DNA damage. Insulin deficient cells are also succumbed to apoptosis, associated with impaired phosphatidylinisitol 3 kinase (PI-3 ) / AKT signalling pathway and decreased Bcl to Bax ratio (36) (Bax is proapoptotic factor but Bcl 2 is antiapoptotic factor).

**Oxidative Stress and Angiotsensin II**

ROS activates the renin-angiotensin system (RAS), leading to increased angiotensin II (Ang II) that causes the cardiomyocyte hypertrophy and apoptosis (37). In rats, RAS is activated despite minimal changes in myocardial loading leading to cardiomyocyte hypertrophy and apoptosis (4).

Myocyte hypertrophy and apoptosis occur by upregulation of any component of the RAS from angiotensinogen to angiotensin receptor level (38). Ang II has four receptors. Both receptor 1 (AT1) and receptor 2 (AT2) cause apoptosis. AT 1 receptor mediated apoptosis may occur secondary to vascular growth and remodelling and AT2 receptor mediated apoptosis oppose cell proliferation. Both the AT1 and AT2 increase the proapoptotic factor Bax but AT2 decreases the antiapoptotic factor Bcl 2. Cardio myocyte apoptosis is initiated by AT1. AT1 activates tyrosin kinase c-src that stimulates phospholipase C (PLC-γ) leading to the formation of diacyle glycerol and activation of protein kinase C (PKC). Increase in the activity of PKC causes the activation of nicotinamide adenine dinucleoside phosphate (NADPH) oxidase and produces superoxide (O2•-), which reacts with nitric oxide (NO) to form peroxynitrate (ONO2). Protein, lipid as well as purine and pyrimidine of DNA are oxidized by ONO2 that stimulates. Apoptosis occurs as a result of P53 mediated elevation in the Bax/Blc 2 ratio and dependent mechanism that results in mitochondrial death (39).

AT2 linked death cascade requires tyrosine phosphatase that reduces phosphorylation state Bcl-2 and upregulation of Bax as well as promotes ceramide synthesis. Elevation of intracellular ceramide proceeds caspase-3 activation and DNA fragmentation (40). It is noted that apoptosis is mainly mediated by activation of caspase-3 but it does not affect necrosis in induced ischaemia/reoxygenation (41).

Moreover, Ang II stimulate the release of aldosterone that also stimulates cardiomyocyte apoptosis by caspase 3 activation (42).

Local Angiotensin II effects are modulated by the function of insulin like growth factor (IGF)- a key factor for cardiac growth and function. In diabetes, IGF-1 is reduced that activates dependent gene regulation and leads to up regulation of Bax, local RAS and accumulation of ROS that causes myocyte death (37). Myocyte apoptosis was increased by two fold without IGF-1, was at 7 and 30 days after the onset of diabetes. After 30 days, myocyte necrosis was severe with loss of 24% of ventricular myocytes and there was 28 % myocyte hypertrophy (37).

**Oxidative Stress and Inflammatory Cytokines**

Sustained hyperglycaemia causes increase in ROS which causes a decrease in NO level that leads to myocardial inflammation and endothelial dysfunction (4). Myocardial inflammation produces large number of cytokines (IL1, IL6 and TNF). Interleukin 1 (IL 1) causes myocyte hypertrophy and decreases the myocyte contractility. Tissue necrosis factor (TNF) leads to myocardocyte apoptosis (43).

TNF mediated cardiac apoptosis is activated by both the extrinsic and intrinsic death pathways. Extrinsic pathway is initiated by ligands that bind to death receptor TNFR1 (TNF receptor1). It has two sequential complexes termed “complex I” and “complex II,” whereas intrinsic pathway is governed by various proapoptotic proteins from mitochondria that lead to increased cytosolic level of cytochrome-c, caspase-3 and caspase-9 so as to restrict Bcl 2 over expression (43).

Engagement of TNFR1 by TNF ligand leads to recruitment of the death domain–containing proteins TRADD and TNFR-interacting protein serine-threonine kinase 1 (RIP1), which then participate in, either by complex I or complex II. TNFR1 also promotes the activation of the cytoprotective transcription factor NF-κB as well
Figure 2: shows apoptosis by the specific cell signal and ATP depletion pathway

as activation of JNK. When NF-êB is activated by complex I, complex II is inhibited by inhibitor c-FLIPL, and the cell survives. However, when c-FLIPL is degraded by JNK, activation of complex II cell death is preceded. Complex II is located in the cytosol, assembled with TRADD and RIP1, where they associate with Fas death domain (FADD) and caspase-8 (43).

The decreased levels of c-FLIPL protein may have allowed for the activation of caspase-8 within complex II as well as the cleavage of Bid to Bid with subsequent mitochondrial release of proapoptotic factors that amplified the activation of the downstream intrinsic pathway (43).

Beside, TNFa mediated apoptosis is also involved in the production of second messenger sphingolipid, including ceramide, that down regulates the expression of Bcl-2 and causes programmed cell death (44).

Oxidative Stress and Reduction of ATP

Oxidative stress decreases the ATP generation which leads to decreased contractile function of the myocyte 15. In diabetes, cardio myocyte ATP reduction causes apoptosis through non signalling pathway. Depletion of ATP leads to loss of normal cellular membrane function, disruption of transmembrane ion gradient and membrane rupture (45) (Figure 2). Both necrosis and apoptosis appear in heart muscle by this process and may contribute to cardiac dysfunction in diabetes, ischaemia and hypertension (45).

Mechanical Stress

Mechanical force produced by haemodynamic load may lead to myocardial cell death and enhances the production of ROS 10. Experiments revealed that isolated papillary muscle exposed to high level of tension for a three hour period caused apoptotic myocyte cell death. Imposition of high load increases O2 consumption and this phenomenon leads to the generation of O2. Increased endogenous O2 production decreases NO release and enhances expression of Fas receptor protein (expression depends upon extent of load imposed on the myocardium) that leads to activate programmed cell death 10. In this way, free radicals are formed during both ischaemia and reperfusion injury (46). Treatment with a No releasing drug (C87-3754) revealed that the myocyte is protected from detrimental effects of overstretching apoptosis by preventing DNA strand breaking. NO may alleviate oxidative stress and thereby protect the heart from cardiac apoptosis (10).

Apoptosis and Myocardial Disarray

Whatever the mechanism for apoptosis, cardio-myocytes apoptosis is obvious in diabetes. It is
said that apoptosis may cause architectural rearrangement of the myocardium, involving side to side slippage of myocytes. Single myocyte cell death has been postulated to occur to allow side by side translocation of cells within the wall. Sliding of the myocyte bundle, however, would need multiple cells death (10). Nevertheless, loss of cardiac myocyte without cardiovascular disease has also been observed in senescent heart in human and animals. In human, a loss of an average of (38) million cells per year may accompany by a reciprocal increase in myocyte volume per nucleus, thereby preserving ventricular wall thickness (45).

Structure properties of the ventricle are determined not only by its myocytes but also by the interstitial connective tissue, which is rich in fibrillar collagen III and I. Collagen III acts as a strut along with the myocyte, it is aligned to branches of collagen fibres course at right angle to connect and align the muscle bundle (group of six or more myocyte). Thus a depletion of this strut may lead to chamber dilatation (45). In the failing heart, this setting is distorted by weakened connective tissue, which disturbs organ integrity, myocyte slippage and ventricular remodeling (33).

In diabetes, increased NO level leads to cardiac fibroblast and vascular smooth muscle apoptosis leading to increase cytokines (11). IL1β causes activation of nitric oxide synthetase (iNOS) that leads to increase the production of ONO2. This can trigger the fibroblast apoptosis which may hamper collagen synthesis (11).

Collagen synthesis is also degraded by cytokines matrix metalloproteinase (MMPs) and this degradation is regulated by other cytokines tissue inhibitor matrix metalloproteinase (TIMPs). In dilated cardiomyopathy and ischaemic cardiomyopathy, excessive collagenolysis may lead to myocyte rearrangement (slippage) that accounts for wall thinning and dilatation, which characterizes end stage heart failure (12, 13, 45). Tissue necrosis factor (TNF) as well as other cytokines and peptide growth factors activate MMPs within the failing myocardium (13).

Thus, apoptosis of cardiac muscle cell and non-cardiac muscle may contribute to develop myocardial disarray in diabetes and may causes functional limitation of heart.

Hypertrophy and Myocardial Disarray
It has been suggested that myocyte hypertrophy and myocardial fibre disarray are important in the pathogenesis of sever congestive heart failure (47). Left ventricular hypertrophy (LVH) was demonstrated in one-third of patients with type II diabetes independent of blood pressure. 4 Left ventricular hypertrophy (4, 35, 48, 49) and abnormal systolic function (35) frequently coexist in diabetic patients. Aortic stiffness and diastolic dysfunction in diabetic patients by increasing end systolic wall stress may contribute to the development of LVH (50) It is revealed that systole of HCM is abnormal, has additional middle or late systolic phase which may occupy over half of the systole, ventricle is nearly empty, generates a pressure twice higher than normal pre-ejection phase. Such isometric contractions provide a power full stimulus to ventricular hypertrophy and alter the contraction induced linear stress that develops the cardiac muscle cells disalignment (25). Myocardial disarray may develop in diabetes secondarily from this abnormal systolic pressure.

Conclusion
Sudden and unexpected death is seen in hypertrophic cardiomyopathy (HCM) due to presence of myocardial disarray. Literature shows that myocyte apoptosis may involve side-to-side slippage of myocytes that leads to myocyte disarray. Whatever the mechanism for apoptosis, cardio-myocytes apoptosis is obvious in diabetes. In addition to that, one third of diabetic patients have hypertrophied hearts due to ventricular dysfunction. Thus there may be a link to develop myocardial disarray in diabetes that may express cardiac dysfunction leading to heart failure. Further studies along these lines are essential.

References


6. Warley A, Powell JM, Skepper JN. Capillary surface area is reduced and tissue thickness from capillaries to myocytes is increased in the left ventricle of STZ diabetic rat. Diabetologia 1995; 38: 413-421.


