A putative role for oxidative stress in pathophysiology of diabetic cardiomyopathy

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Objectives
Cardiovascular disease associated with diabetes remains the leading cause of morbidity and mortality worldwide. There is a growing scientific and public interest in connecting oxidative stress as a cause of endothelial dysfunction associated with pathological conditions such as diabetes mellitus (DM). Free radicals' scavengers play a pivotal role in maintaining homeostasis. We investigated potential risk factors and the role of oxidative stress in the pathophysiology of diabetic cardiomyopathy.
Patients and methods
Eighty type 2 DM patients along with 65 normal healthy volunteers were recruited for this study. We calculated BMI, and measured arterial blood pressure. We measured glycosylated hemoglobin (HbA_{1C}), lipid profile levels, catalase, nitric oxide, the enzymes superoxide dismutase, and malondialdehyde in plasma.
Results
We found that the mean BMI (32.63±3.42), HbA_{1C} (8.07±1.39), malondialdehyde (1007.21±299.341), and nitric oxide (6.79±1.95) were significantly higher in the patient group compared with the control group. On the other hand, superoxide dismutase (2.97±0.69) and catalase (35.44±10.56) in diabetic patients were significantly lower compared with controls.
Conclusion
Our results confirm the role of oxidative stress in pathophysiology of DM. This suggests that antioxidants may have a putative therapeutic and a prognostic role in diabetic cardiomyopathy.

Keywords:
diabetic cardiomyopathy, diabetic mellitus, oxidative stress

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Introduction

Diabetes mellitus (DM) is a major public health problem in Egypt as a part of the worldwide diabetes pandemic. There were over 7.8 million cases of diabetes in Egypt in 2015, and it comes in the eighth order, which is further amplified by a rise in obesity rates, a high rate of consanguinity, and the presence of other variables of the insulin resistance syndrome [1].

The WHO has estimated that 415 million people have diabetes in the world, with more than 35.4 million people in the Middle East and North Africa Region; by 2040 this will rise to 72.1 million [2]. During the past three decades, the tremendous surge in socioeconomic growth has considerably influenced the lifestyle of the people. Epidemiological studies showed an increase in the prevalence of DM [3]. A study in USA has shown that there is significant increase in the prevalence, which became about 30% in 2011 [4].

Cardiomyopathy is a heart muscle disorder that affects particularly ventricular systolic and diastolic functions as a result of structural and functional alterations in the ventricles [5].

There is an increased incidence of heart failure in diabetic patients, with an estimated rate of four to five folds compared with the nondiabetic patients [6]. The mechanisms leading to diabetic cardiomyopathy are undefined [7].

The excess production of highly reactive free radicals, due to hyperglycemia, causes oxidative stress, through several mechanisms, as well as increased intracellular formation of advanced glycation end products. Such products enhance the progression of diabetes and its cardiovascular complications [8].

Malondialdehyde (MDA) is an indicator of cell and tissue damage, and lipid peroxidation end-product [9]. Nitric oxide (NO) is important for blood vessel

dilatation, neurotransmission, immune defense, and apoptosis. NO is considered as a free radical that, in patients with chronic heart disease, can lead to vasodilatation and dysfunction of the endothelium [10].

The objective of this study is to evaluate risk factors of cardiomyopathy and the role of oxidative stress in diabetic patients through demographic data, anthropometric, and biochemical profiles.

Patients and methods

This study was approved by Faculty of Medicine, Helwan University Research Ethics Committee. Eighty non-insulin-dependent diabetics with cardiomyopathy were recruited in this study from Endocrine Outpatient Clinic, Helwan University Hospitals, with age ranging from 52 to 74 years, with a mean age of 58.46±6.02 years; 48 (60%) were male with a mean age of 59.32±6.10 years and 32 (40%) were female with a mean age of 56.06±5.68 years. Sixty-five apparently healthy volunteers matched for age and sex, without any history of heart and systemic disorders, were included in the study as the control group. Both groups provided signed informed consent.

A comprehensive sheet was fulfilled from all participants to collect demographic data including age, sex, special habits, and duration of diabetes. Height and weight were measured and the BMI was calculated in kg/m^2. Arterial blood pressure was measured with a sphygmomanometer after 5 min of rest.

Venous blood samples (10 ml) were collected using anticoagulant (EDTA) for the analysis of plasma MDA where it was quantified following protocol of Dahle *et al*. [11], and without EDTA for the analysis of serum NO, which were assayed based on Green *et al*. method [12]. Evidence of cardiac abnormalities by Standard clinical, ECG, and echocardiographic criteria was used for diagnosis.

We used conventional two-dimensional echo cardiography, in addition to pulsed wave Doppler, and the following echocardiographic criteria were used for diagnosing diastolic dysfunction:

(1) A 'preserved' ejection fraction (defined as left ventricular ejection fraction ≥50%).
(2) Left atrial volume index of at least 34 ml/m^2 body surface area.
(3) Left ventricular mass index of at least 115 g/m^2 for males and of at least 95 g/m^2 for females.
(4) *E*/*e*′ of at least 13 and a mean septal and lateral wall *e*′ wave less than 9 cm/s.

An ejection fraction less than 40% was used for diagnosing systolic dysfunction [13].

The normal range for total cholesterol (CHOL) was up to 200 mg/dl [14]. The normal range for high-density lipoprotein CHOL concentration was 30–60
mg/dl in males and 40–70 mg/dl in females [15]. Triglycerides (TG) were measured using standard colorimetric analytical methods. The normal range for TG was up to 200 mg/dl [16].

However, the low-density lipoprotein cholesterol concentration (LDL-cholesterol) was calculated by the Friedewald formula [17]. Glycosated hemoglobin (HbA_{1C}) was measured after preparing the hemolysate, where the labile fraction is eliminated; hemoglobins are retained by a cationic exchange resin. Hemoglobin $A1_C$ is specifically eluted after washing away the hemoglobin A1a+b fraction, and is quantified by direct photometric reading at 415 nm [18]. The normal values were in the range of 4.2–6.2%.

The serum catalase (CAT) enzyme activity was determined by spectrophotometric method, as CAT catalyzes the breakdown of H_2O_2. The remaining H_2O_2 in the reaction mixture facilitates the coupling of this reaction and it is monitored at 520 nm. The decrease in absorbance is proportional to the activity of CAT [19]. CAT activity was expressed as U/ml. The serum superoxide dismutase (SOD) activity was determined at 37°C by the xanthine/xanthine oxidase system that generates the superoxide anions. The presence of SOD resulted in the decrease of superoxide anions yielding less colorimetric signal at 490 nm. SOD was expressed as U/ml Oyanagui [20].

Statistical analyses

All results were expressed as mean and SD. Statistical analyses were evaluated by using statistical package for the social sciences (version XIII; SPSS Inc., Chicago, Illinois, USA). Differences at the level of 0.05 or less were considered statistically significant.

Results

The cardiomyopathy patients had a mean duration of DM of 16.56±3.63 years. The BMI was 32.63±3.42 kg/m^2 for patients and 28.25±5.48 kg/m^2 for controls. The

mean systolic and diastolic blood pressures for the patient group were 140.80±10.58 and 114.54±8.10 mmHg, respectively, and for the control group they were 91.48±5.80 and 71±7.44 mmHg, respectively. On the other hand, we found that the diabetic patients had poor blood glucose control, as indicated by higher glycated hemoglobin of 8.07±1.39 and 4.84±0.65 in the control group (Table 1). There was hyperlipidemia in cardiomyopathy patients where the total CHOL, TG, and the LDL were higher than the normal values (Table 2).

The mean levels of MDA were found to be 423.16 ±74.24 in controls and 1007.21±307.38 in diabetic group, with a significant increase in the patient group (t: 11.734 and P=0.0001). The mean levels of NO were found to be 3.53±1.16 and 6.79±1.95 in control and diabetic groups, respectively. The increase in the mean level of NO in patients was found to be significant (t: 9.323 and P=0.0001). Similarly, the mean level of SOD in the diabetic group (2.35±0.78) was significantly low (P=0.020) when compared with the control group individuals (4.94±1.22). The mean levels of CAT were also found to be significantly lower (36.34±4.24) (P=0.0001) in the disease group compared with the control group individuals (44.11±3.95) (Table 3).

Discussion

Our results showed that in addition to elevated BMI, HbA_{1C}, and lipid profile, patients with diabetic cardiomyopathy had increased NO and MDA production and reduced SOD and CAT enzymes. This is an indication of oxidative stress associated with hyperglycemia and hyperlipidemia that may contribute to the development of diabetic cardiomyopathy.

There was a significantly higher BMI, HbA_{1C}, significantly higher CHOL, LDL-C, and TG in diabetics compared with controls. It has been suggested that carbohydrate and lipid metabolic abnormalities, such as hyperglycemia and hyperlipidemia, may lead to the development of cardiac dysfunction in DM [21]. Fiorentino *et al.* [22] stated that the prolonged hyperglycemia causes vascular damage through oxidative stress. On the other hand, abnormalities in lipid metabolism are correlated with the severity of the myocardial dysfunction in cardiomyopathy [23]. These results align with the animal studies that showed that hyperglycemia is associated with hypercholesterolemia and hypertriglyceridemia in rats [24]. Our results also conform with results of Diabetes Control and Complications trial that demonstrated that tight control of blood glucose is effective in reducing clinical complications [25].

Table 1 Distribution of the descriptive data of age, diabetes BMI, systolic blood pressure, diastolic blood pressure, and glycosylated hemoglobin in the control and diabetic patient groups

	Control group	Diabetic patients
Age (years)	57.23±5.23	58.46±6.02
BMI (kg/m^2)	28.25±5.48	32.63±3.42*
Systolic blood pressure (mmHg)	114.54±8.10	140.80±10.58*
Diastolic blood pressure (mmHg)	71±7.44	91.48±5.80*
Glycosated hemoglobin (%)	4.84±0.65	8.07±1.39*

*Statistically significant differences at the level of 0.05 or less.

Table 2 Distribution of the lipid profile parameter values in the control and diabetic patient groups

	Control group	Diabetic patient group
Total cholesterol (mg/dl)	3.97±0.84	6.03±1.02*
Triglycerides (mg/dl)	2.80±1.51	4.93±1.20*
Low-density lipoprotein (mmol/l)	1.83±1.29	2.54±1.47*
High-density lipoprotein (mmol/l)	1.81±0.48	2.98±0.91*

*Statistically significant differences at the level of 0.05 or less.

Table 3 Distribution of the quantitative levels of malondialdehyde, nitric oxide, catalase, and superoxide dismutase parameters in the control and diabetic patient groups

	Control group	Diabetic patient group
Malondialdehyde (nmol/l/dl)	423.16±74.24	1007.21±307.38*
Nitric oxide (μmol/l/l)	3.53±1.16	6.79±1.95*
Catalase (U/ml)	44.12±7.34*	35.44±10.56
Superoxide dismutase (U/ml)	4.22±1.14*	2.97±0.69

*Statistically significant differences at the level of 0.05 or less.

We observed that the MDA level is significantly higher in the patient group as compared with the control group. MDA can be a useful marker of the severity of the tissue damage [9]. Its high level is an indicator of lipid peroxidative damage [26]. Lipid peroxidation leads to production of mutagenic lipid epoxides, lipid hydroperoxides, lipid alkoxyl, and peroxyl radicals and enols [27]. The release of these free radicals may cause cellular alteration leading to loss of membrane functions and myocyte architecture that may contribute to the pathophysiology of diabetic cardiomyopathy [28].

NO levels were significantly raised in the patient group compared with the controls in our study. NO is a free radical, and in patients with cardiovascular disease it leads to vasodilatation and dysfunction of the endothelium [10]. Excess of NO may cause cell injury and generate cytotoxic species, which may play an active role in atherosclerosis in endothelial cells and facilitate platelet aggregation and plaque formation [29]. Raised NO levels could thus indicate a direct role in the muscle oxidative stress as it is directly released from the endothelial cells [30]. NO plays a critical role in the regulation of integrated cardiac and vascular function and homeostasis [31].

In this study, the mean levels of SOD and CAT in the diabetic group were significantly low when compared with the control group, indicating the role of oxidative-stress-mediated tissue injury and lipid peroxidation in diabetic cardiomyopathy patients. The enzymatic antioxidant such as SOD and CAT that catalyze reactions to neutralize free radicals and reactive oxygen species (ROS) were significantly decreased compared with the control group. SOD is an enzyme that catalyzes the breakdown of the superoxide anion into oxygen and hydrogen peroxide, whereas CAT enzyme catalyzes the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. These enzymes are present in almost all aerobic cells and in extracellular fluids. They act as free-radical scavengers through several mechanisms, and their reduction may result in cell damage [32,33]

To summarize, our results showed that in addition to elevated BMI, HbA_{1C}, and lipid profile, patients with diabetic cardiomyopathy had increased NO and MDA production and reduced SOD and CAT enzymes. These results indicate the presence of oxidative stress associated with hyperglycemia and hyperlipidemia in diabetic cardiomyopathy. Increased ROS production in the diabetic heart contributes to the development and progression of diabetic cardiomyopathy [34]. Increased ROS generation may activate maladaptive signaling pathways, which may lead to cell death and could promote abnormal cardiac remodeling, which ultimately may contribute to the characteristic morphological and functional abnormalities that are associated with diabetic cardiomyopathy [7,35].

Conclusion

Our results demonstrate a putative role for oxidative stress represented by excess NO production and reduced scavenging enzyme activity in the pathophysiology of diabetic cardiomyopathy. Antioxidants may have a putative therapeutic and prognostic role in diabetic cardiomyopathy.

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Conflicts of interest

There are no conflicts of interest.

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