Original article

TNF-α correlates oxidative stress-induced cardiomyopathy: A comparative study among Indian male and female diabetic patients

Pradeep Bhatt [1], Deepak Sharma [2]

[1]Research Scholar, Clinical Biochemistry Department, Sri Venkataeshwara University, Gajraula U.P. India.,
[2]Professor, School of Life Sciences, Jawaharlal Nehru University, New Delhi – 110067.
Corresponding author : Pradeep Bhatt

Abstract:

Diabetic cardiomyopathy (DCM) is common disorder of the heart muscle and it contributes significantly to cardiovascular related death in the diabetic population. Oxidative stress plays a significant role in the development of diabetic cardiomyopathy. Tumour necrosis factor (TNF-α) which is increasingly expressed in the failing heart and in blood may stimulate oxidative stress.

Objective: we investigated the relationship of TNF-α with oxidative stress in well and poorly controlled type-1 & type-2 diabetic cardiomyopathic Indian male and female subjects.

Material and Methods: We measured plasma TNF-α, blood HbA1C & oxidative markers (MDA, SOD,) in the remnants samples, which come in lab for various investigations and collect ECHO data for the same subjects from various heart clinics.

Keywords: Diabetic cardiomyopathy, Oxidative stress, TNF-α, Inflammation, Hyperglycemia.

Introduction:

Diabetic cardiomyopathy (DCM), one of the leading cardiovascular complications in diabetic population, has gained momentum due to its subsequent heart failure and increased mortality. It is a different clinical entity of diabetic heart muscle that explain diabetes associated changes in the structure and function of the myocardium in the absence of coronary artery disease, hypertension and valvular diseases (1,2,3). Over the last decades, various evidence from both clinical data and animal model shows that diverse mechanisms are involved in the development of diabetic cardiomyopathy, including alteration in substrate metabolism, oxidative stress induced damage, cardiac inflammation and fibrosis (4,5,6). Among these abnormalities, the inflammatory response and oxidative stress play a key role in the onset and progression of diabetic cardiomyopathy. Chronic inflammation could directly or indirectly cause cardiac tissue injury such as myocardial fibrosis, apoptosis and necrosis, which certainly leads to left ventricular (LV) diastolic and then systolic dysfunction (7,8).

Oxidative stress plays a vital role in DCM development. Oxidative stress is defined as an excessive formation or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (9). Under normal physiological conditions, continuous generation of ROSis countered by antioxidants defense mechanism (10). Excessive ROS production is known to be associated with direct cellular damages of protein and DNA, activation of apoptosis and ROS mediated cell death which could promotes abnormal cardiac remodeling, and ultimately leads to the characteristic morphological and functional

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abnormalities, associated with DCM (11, 12).Since free radical cannot be measured directly in humans, indirect oxidative markers and their activity is being used to assess the oxidative stress. The most widely used markers are malondialdhyde, carbonyl protein and antioxidant like SOD, GPX, GSH for the assessment of oxidative stress in diabetic cardiomyopathic subjects.

Tumour Necrosis factor-α (TNF-α) is pleiotropic pro-inflammatory cytokiene that is produced essentially by activated macropahage. TNF-α has been shown to increase expression of adhesion molecules, stimulate the release of endothelial cytokiene and nitric oxide (NO), increase vascular permeability, decrease contractility and induce a prothrombotic state (13, 14). A potentially important stimulus for increased oxidative stress in the failing myocardium is believed to be due to exposure to inflammatory cytokiene, such as TNF-α, (15, 16) and shown to stimulate free radical production (17, 18). Animal experiment studies have reported the over-expression of chronic inflammation of TNF-α leads to myocarditis, ventricular systolic dysfunction, ventricular dilation and hypertrophy, myocardial fibrosis and apoptosis all leading to high mortality in these animals (19, 20). In patients with chronic heart failure (CHF) there is a direct correlation between TNF-α levels and symptom severity and mortality rate of DCM (21).Since type-II diabetic females are found to be more vulnerable for oxidative induced cardiomyopathy, it would be of great significance to investigate the relationship of TNF-α with oxidative parameters in such patients.

Therefore, in the present study attempts were made to compare the interrelationship if any, between TNF-α and oxidative markers viz, HbA1C, MDA, SOD in both well and poorly controlled Indian cariomyopathic male and females.

Material and methods:

The present study was performed in the remnant fasting blood samples received from the type-1 and type-2 diabetic /diabetic cardiomyopathic subjects come to the various clinics for the routine health check up. We collaborate with various heart clinics for the remnant fasting blood samples and ECG & ECHO data for our thesis work. Exclusion criteria included subjects with complications of diabetes mellitus like retinopathy, nephropathy, and subjects in antioxidants therapy, smoker and hypertensive patients. Samples from the patients coming for routine investigations were collected in EDTA and plain vacutainer with clot activator. After completed clinical investigation, each sample was immediately processed for separating Plasma, serum and making hemolysate. For glycosylated hemoglobin separate EDTA samples were used. All clotted samples were centrifuged at 3000 RPM for 10 – 15 minutes at 4^0C. Serum was separated and stored into three different eppendrof at – 80^0C. EDTA plasma, RBCs was washed three times with 0.85 % sodium chloride solution and again centrifuge at 3000 RPM for 10 – 15 minutes at 4^0C, and cold HPLC grade water was added to make hemolysate. After 5 – 10 minutes samples were centrifuged at 5000 RPM for 10 – 15 minutes at 4^0C. Supernatant was discarded and hemolysate was collected into 4 different eppendrof. Plasma and hemolysate was stored at – 80^0C, whereas glycosylated hemoglobin EDTA samples were stored at 4 ^{0}C. Subjects were divided into the following category.

01. Normal subjects without diabetes (Number = 20).
02. Indian male controlled hyperglycemia with T1DM +cardiomyopathy (Number = 20)

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03. Indian male poorly controlled hyperglycemia with T1DM + cardiomyopathy (Number = 20).
04. Indian female controlled hyperglycemia with T1DM + cardiomyoapthy (Number = 20)
05. Indian female poorly controlled hyperglycemia with T1DM + cardiomyopathy (Number = 20)
06. Indian male controlled hyperglycemia with T2DM + cardiomyoapthy (Number = 20)
07. Indian male with poorly controlled hyperglycemia with T2DM + cardiomyopathy(Number = 20)
08. Indian female controlled hyperglycemia with T2DM + cardiomyopathy (Number = 20).
09. Indian female with poorly controlled hyperglycemia with T2DM + cardiomyopathy (Number = 20).

Parameter assayed in Serum:

Tumour Necrosis factor- α (TNF-α) (Meager.et.al., 1989) : Serum TNF-α levels in all subjects were measured by ELISA based kits (eBiosciences, San Diego, CA).

Parameter assayed in Blood:

Glycosylated Hemoglobin (HbA1C) Assay–HbA1C levels were assayed according to the method of Meyer.et.al.,1983. Whole blood glycosylated hemoglobin concentration were performed by high performance chromatography (HPLC) using a fully automated Bio-rad machine.

Parameters assayed in hemolysate:

Blood MDA Assay – MDA levels were assayed according to the method of Jain.et.al.,1989.

Superoxide dismutase (SOD) – SOD levels were assayed according to the method of Sun.et.al.,1988.

ECG and 3D ECHO –ECG done on automated 12 lead BPL ECG machine and 3 dimensional ECHO done on voluson E-8 and Logiq S-8 by GE Healthcare. Data of both poorly & well controlled male & female diabetic patients with cardiomyopthy with collected from various cardiac centers.

Statistical analysis:

The results were expressed as (mean ±SD) and analyzed statistically, the difference between the results of patients and control group were assessed by students t test. Level of Significance was considered only at P value less than 0.001. Correlation between the glycemic or oxidative marker and cardiomyopathic marker were performed by Spearman correlation analysis (r-value).

The regression analysis was also performed for these values.

Results:

Parameters	Control Subjects	Type I Diabetic Cardiomyopathy				Type II Diabetic Cardiomyopathy			
	♂♀	Male ♂		Female ♀		**Male ♂**		**Female ♀**	
		WC	**PC**	WC	**PC**	WC	**PC**	WC	**PC**
TNF-α (pg/ml)	2.0± 0.2	1.0 ± 0.4	**4.6 ± 0.3 (P<0.001)**	2.2± 0.2	**5.1 ± 0.4 (P<0.001)**	2.2 ± 0.3	**4.4 ± 0.4 (P<0.001)**	2.6 ± 0.4	**5.5 ± 0.3 (P<0.001)**
HbA1C (%)	5.4±0.2	6.4±0.2	**12.0 ± 0.8 (P<0.001)**	6.5±0.5	**9.0 ± 1.0 (P<0.001)**	6.0±0.2	**13.0 ± 0.5 (P<0.001)**	6.0±0.2	**9.5 ± 0.8 (P<0.001)**
MDA	1.10± 0.31	1.45 ± 0.11	**2.52 ± 0.34 (P<0.001)**	1.32 ± 0.14	**2.60 ± 0.30 (P<0.001)**	1.50 ± 0.18	**2.55 ± 0.26 (P<0.001)**	1.44 ± 0.11	**2.65 ± 0.17 (P<0.001)**
SOD	1477 ± 110	1250 ± 102	**1010 ± 55 (P<0.001)**	1120 ± 81	**822 ± 44 (P<0.001)**	1311 ± 84	**880 ± 52 (P<0.001)**	1290 ± 64	**910 ± 44 (P<0.001)**
ECHO (LVM) gm	130 ± 19	139 ± 20	**238± 15 (P<0.001)**	145 ± 18	**190 + 12 (P<0.001)**	167 + 17	**264 + 20 (P<0.001)**	150+ 11	**200 + 19 (P<0.001)**

WC – Well controlled Glycemia **PC**– Poorly controlled Glycemia

TNF-α level in serum:

It is evident from the table I that levels of TNF-α in serum higher in poorly controlled type-II diabetic cardiomyopathic male and females, compared to type- I male and females respectively. Our data shows that in levels of TNF-α in serum higher in female type-I and type-II diabetic cardiomyopathic subjects as compared to male.

Correlation studies:

TNF-α Vs HbA1C:

Correlation studies showed a significant positive correlation between TNF-α and HbA1C in type-1 & type-2 poorly controlled diabetic cardiomyopathic subjects. The correlation coefficient in type-1 poorly controlled hyperglycemia induced diabetic cardiomyopathic female subjects was higher (r = 0.81), compared to male (r = 0.74). Similarly correlation coefficient in type-II female subjects was higher (r = 0.76) (figure-02), compared to male (r = 0.69) (figure-01).

TNF-α Vs MDA:

Correlation studies showed a significant positive correlation between TNF-α and MDA in type-1 & type-2 poorly controlled diabetic cardiomyopathic subjects. The correlation coefficient in type-1 poorly controlled hyperglycemia induced diabetic cardiomyopathic female subjects was higher (r = 0.82), compared to male (r = 0.77). Similarly correlation coefficient in type-II female subjects was higher (r = 0.77) (figure-04), compared to male (r = 0.70) (figure-03).

TNF-α Vs SOD:

Correlation studies showed a significant negative correlation between TNF-α and superoxide dismutase (SOD) in type-1 & type-2 poorly controlled diabetic cardiomyopathic subjects. The correlation coefficient in type-1 poorly controlled hyperglycemia induced

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diabetic cardiomyopathic female subjects was higher (r = - 0.80), compared to male (r = - 0.76). Similarly correlation coefficient in type-II female subjects was higher (r = - 0.82) (figure-6), compared to male (r = - 0.78) (figure-5).

TNF-α Vs LVM (ECHO):

Correlation studies showed a significant positive correlation between TNF-α and left ventricular mass (LVM) in type-1 & type-2 poorly controlled diabetic cardiomyopathic subjects. The correlation coefficient in type-1 poorly controlled hyperglycemia induced diabetic cardiomyopathic female subjects was higher (r = 0.80), compared to male (r = 0.73).

Similarly correlation coefficient in type-II female subjects was higher (r = 0.77) (figure-08), compared to male (r = 0.71) (figure-07).

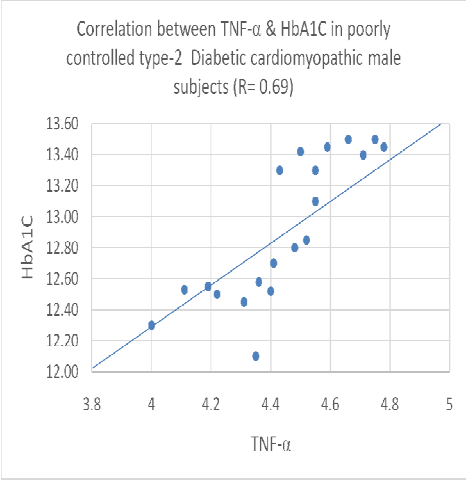

Figure – 01.

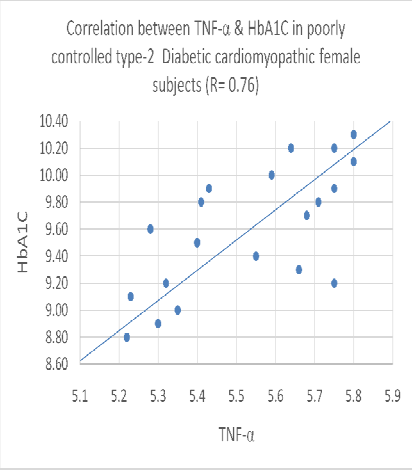

Figure – 02.

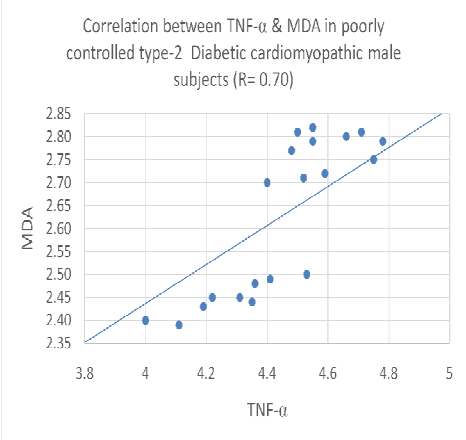

Figure – 03.

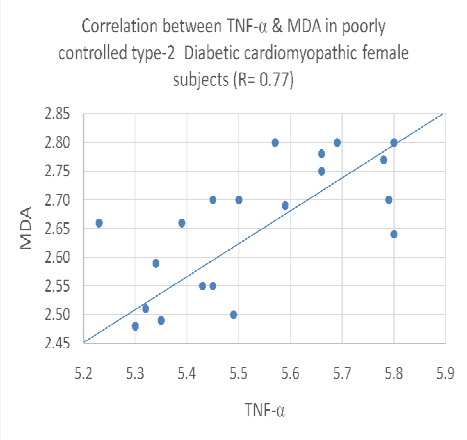

Figure-04.

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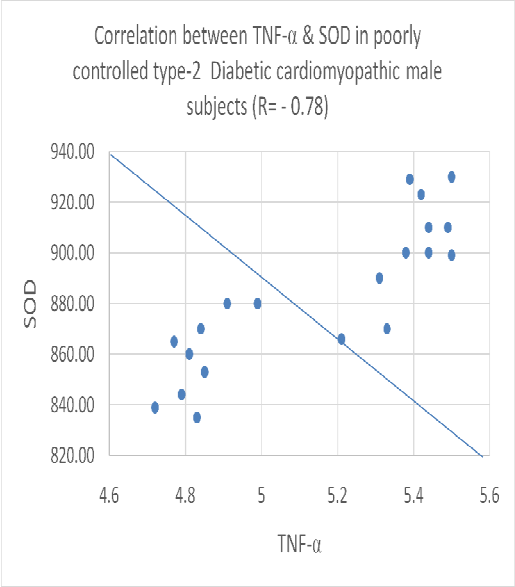

Figure-05.

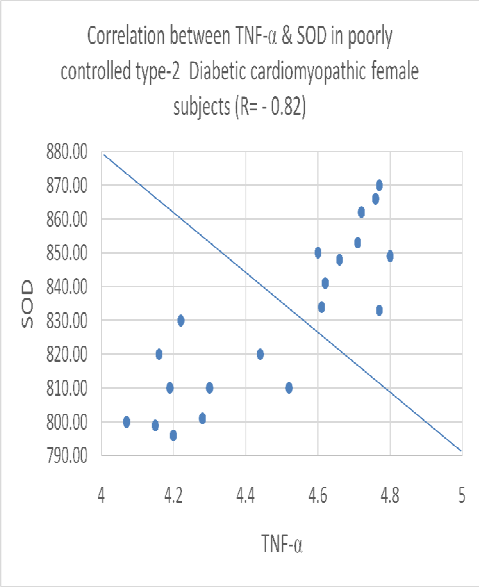

Figure-06.

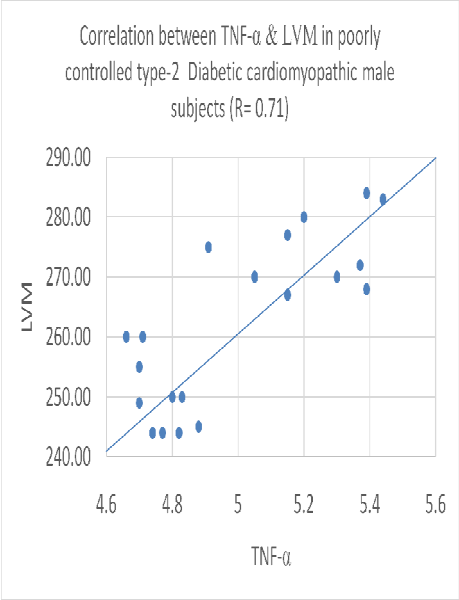

Figure -07.

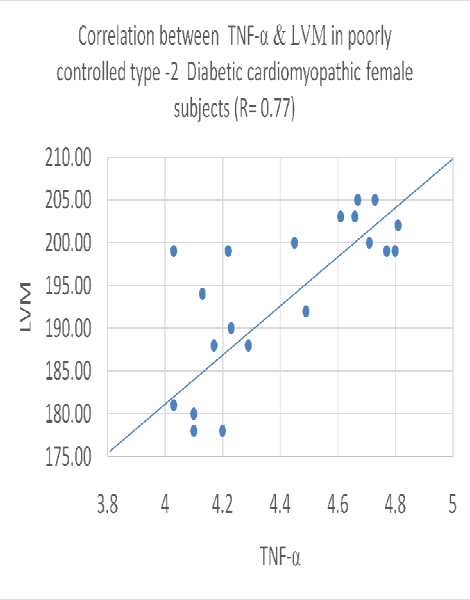

Figure – 08.

Discussion:

Our data were able to demonstrate that oxidative stress and pro-inflammatory cytokines are associated with well and poorly controlled type-1 & type-2 diabetic cardiomyopathic Indian male and female subjects. In particular increased serum levels TNF-α seem to be correlated with oxidative stress in poorly controlled type-1 & type-2 diabetic cardiomyopathic subjects. Our data provide clinically important information on systemic immune abnormalities in Indian male & female diabetic cardiomyopathic subjects. There is increasing evidence that inflammation is involved in the pathophysiology of heart failure and diabetes (28, 29, 30). Inflammation has become one of the central themes in the pathogenesis of systolic heart diseases over the past

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decade. So far there have been few data on participation of inflammatory factor in the development of diastolic dysfunction of diabetic patients.

Pro-inflammatory cytokienes are capable of modulating cardiovascular function by various mechanisms. It is now known that virtually every nucleated cell type in the myocardium including the cardiac myocyte, is able to secrete pro-inflammatory cytokienes in response to carious myocardial damage or stressor by poorly controlled hyperglycemia. The pro-inflammatory TNF-α induces cardio-protective effects and causes apoptosis (31). The development of progressive cardiomyocyte apoptosis by diabetes plays a critical role on the diabetic cardiomyopathy and the adverse cardiac remodeling that occurs in the setting of sustained inflammation.

Our data showing a positive & direct correlation between increased serum TNF-α level and oxidative stress in poorly controlled diabetic cardiomyopathic male & female subjects is surfaced as a novelty of present study. Oxidative stress is the critical and central mediator involved in diabetes induced myocardial cell death (32). Oxidative stress is also involved in necrotic cardiomyocyte death since it leads to mitochondrial calcium overloading, opening of the mitochondrial permeability transition pore, mitochondrial swelling, and ATP depletion, which triggers necrotic cell death (33, 34). In addition, lipid peroxidation may also contribute in cardiomyocytes necrosis (34). A potentially important stimulus for increased oxidative stress in the myocardium is exposure to inflammatory to pro-inflammatory cytokienes, such as TNF-α which is increasingly present in the failing myocardium but not in the healthy heart (15, 35) and which has the ability to stimulate free radical production (18, 36). Few studies reported that the myocardial over- expression of TNF-α causes ventricular dilation, ventricular dysfunction and interstitial fibrosis suggesting the local expression of TNF-α along with oxidative stress markers in the heart plays a causal role in the pathogenesis of DCM (37, 38). Tsutamoto.et.al.2001 reported that transcardiac increase in TNF-α along with ox-LDL, an oxidative marker was observed in patients with DCM and mild congestive heart failure (CHF), and suggesting that TNF-α is produced in myocardial tissue and the source of increased plasma TNF-α is partly the failing heart. All these studies is not only reported that oxidative stress induced by the local production of TNF-α in the heart may cause left ventricular dysfunction in patients with DCM but also confirming the hypothesis that TNF-α correlates with oxidative stress in diabetes induced cardiomyopathy.

Inflammatory stress markedly exacerbated lipid accumulation in cardiac blood vessels and cardiac collagen deposition, which contributed to the progression of cardiac fibrosis (40). Increased left ventricular mass (LVM) is indicator of cardiac fibrosis in this study. Our data showed that increased LVM is directly correlated with inflammatory marker (TNF-α) induced oxidative stress in the poorly controlled type-1 & type-2 diabetic cardiomyopathic male & female subjects. Recently, many studies reported that inflammatory stress plays an essential role in cardiac fibrosis. Clinical studies demonstrated that myocardial tissue from patients with cardiac fibrosis exhibited the activation of nuclear factor-kappa B (NF-kB) and TNF-α and increased NF-kB and TNF-α regulated gene expression (07). Dyslipidemia induced inflammatory stress was also strongly associated with the progression of cardiac fibrosis or high LVM (41). In the rat model

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Qin.et.al.,(2010) demonstrated that lipid lowering agent inhibited cardiac fibrosis and reduced LVM & serum TNF-α levels by reducing total cholesterol and matrix metalloproteinase-9. The diastolic dysfunction due to dyslipidemia induced inflammatory stress leads to progressive cardiac fibrosis, impaired calcium handling in the heart and thus to contractile dysfunction and increased mitochondrial and endoplasmic reticulum stress contributing to the reduced cardiac energy load (42, 43). All these studies directly or indirectly strengthen the our hypothesis that inflammatory stress (Increased TNF-α level) is associated with changes in ECHO parameters, which are the signs of diabetic cardiomyopathy.

Conclusion:

Pro-inflammatory cytokiene correlates with oxidative stress in poorly controlled type-1 and type-2 diabetic cardiomyopathic Indian male and female subjects. This indicates that inflammatory mediator TNF-α-induced oxidative stress plays a vital role in the development cardiomyopathy in poorly controlled type-I and type-II female diabetic patients as compared to male.

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