

INDIAN FEMALE ARE MORE VULNERABLE FOR OXIDATIVE STRESS INDUCED CARDIOMYOPATHY: A CASE STUDY**Pradeep Bhatt*¹ and Deepak Sharma²**

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ABSTRACT

Oxidative damage is known to play an important role in the pathogenesis of diabetic cardiomyopathy. Since there is no gender based study, we aimed to evaluate the role of oxidative stress in poorly & well controlled diabetic cardiomyopathy in Indian male and female subject. Diabetic cardiomyopathic male & female subjects were categorized into 09 groups and thus blood and serum samples were examined for glycemic marker (HbA1C), oxidative marker (MDA, PCG), antioxidants (SOD, CAT, GSH, GPX), ECG and echocardiographic parameters. **Results:** When compared, poorly controlled diabetic group with well controlled diabetic group former showed significant cardiomyopathic changes. Female had significantly lower levels of SOD, GPX & GSH and significantly higher HbA1C, MDA, carbonyl protein ($P < 0.001$) than male subjects and well

controlled glycemic subjects. ECG & ECHO data were significant correlation with oxidative markers in type-1 & type 2 diabetic cardiomyopathic Indian male and female subjects.

Conclusion: Our data demonstrates that type-2 diabetic females are more prone for cardiomyopathy changes as compared to age matched males.

KEYWORDS: Diabetic cardiomyopathy, Oxidative stress, Hyperglycemia.

INTRODUCTION

Diabetes mellitus (DM), long considered a disease of major significance to world health, is now taking its place as one of the main threats to human health in the 21st century (Zimmet.2000). The international Diabetes Federation estimates that the number of diabetic

patients in India more than doubled from 19 million in 1995 to 40.9 million in 2007. Over the past 30 years, the prevalence of diabetes has increased to 12 -18% in urban India and 3-6% in rural India with significant regional variation. These rates in India are 50 – 80% higher than china (10%) (Ramchandran & Snehlata.2009). The aetiology of diabetes in India is multifactorial and includes genetic factors coupled with environmental influences such as obesity, food habits, lack of exercises, and stress associated with rising living standards, steady urban migration, and lifestyle changes. In India, the steady migration of people from rural to urban areas, the economic boom, and corresponding change in life-style are believed to be important factor in India for the diabetic population. Diabetic cardiomyopathy, one of the leading cardiovascular complications in diabetic subjects, has gained much interest due to its subsequent heart failure and eventually increased mortality. Over the last decades, accumulating evidence from both clinical and experimental data shows that diverse mechanisms are involved in the development of diabetic cardiomyopathy, including microangiopathy, alteration in substrate metabolism, oxidative damage, cardiac inflammation and fibrosis (Falcao &Leite.2012, Stratmann & Tschoepe.2011 & Lorenzo.et.al.,2011). Chronic inflammation could directly and indirectly cause cardiac tissue injury such as myocardial fibrosis, necrosis and apoptosis, which inevitably leads to left ventricular (LV) diastolic and then systolic dysfunction. Hyperglycemia-induced oxidative stress is a major risk factor for the development of micro-vascular pathogenesis in the diabetic myocardium, which result in myocardial cell death, hypertrophy, fibrosis, abnormalities of calcium homeostasis, and endothelial dysfunction (Fang.et.al.,2004, Cai & Young.2003 & Li.et.al.,2005). Although these pathogenic factors cause diabetic cardiomyopathy probably via a different mechanisms (Cai & Kang.2001, Diwan.et.al.,2003 & Ligeti.et.al.,2006), but their major contribution to diabetic cardiomyopathy is believed to be oxidative stress (Brownlee.2005), which is derived directly from these pathogenic factors or indirectly from metabolic intermediates caused by these factors, such as the formation of advanced glycation end-products (AGEs) and production of cytokine or peptides, such as angiotensin-II (AT-II). An area of Clinical Biochemistry diabetes & its complication like Cardiomyopathy, retinopathy, nephropathy and neuropathy are still answered. In this study we tried to investigate reasons why cardiomyopathy occurred in type –I and 2 diabetic people other than hypertensive patients and what are the factors responsible for this in addition to the relationship if any, between diabetic cardiomyopathy and Indian male and female population.

MATERIAL AND METHODS

The present study was performed on the remnant fasting blood samples collected from the type-1 and type-2 diabetic /diabetic cardiomyopathic patients come to the various clinics for the routine health check up. We collaborate with various heart clinics for the blood samples and ECG & ECHO data for further analysis. Subjects with complications of diabetes mellitus like retinopathy, nephropathy, and subjects in antioxidants therapy, smoker and hypertensive patients were excluded. 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 4⁰C. Serum was separated and stored into three different eppendrof at – 80⁰C. EDTA plasma, RBCs was washed three times with 0.85 % sodium chloride solution and again centrifuge at 3000 RPM for 10 – 15 minutes at 4⁰C, 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 4⁰C. Supernatant was discarded and hemolysate was collected into 4 different eppendrof. Plasma and hemolysate was stored at – 80⁰C, whereas glycosylated hemoglobin EDTA samples were stored at 4⁰C. Subjects were divided into the following nine categories.

01. Normal subjects without diabetes (Number = 20).
02. Indian male controlled hyperglycemia with T1DM + cardiomyopathy (Number = 20)
03. Indian male poorly controlled hyperglycemia with T1DM + cardiomyopathy (Number = 20).
04. Indian female controlled hyperglycemia with T1DM + cardiomyopathy (Number = 20)
05. Indian female poorly controlled hyperglycemia with T1DM + cardiomyopathy (Number = 20)
06. Indian male controlled hyperglycemia with T2DM + cardiomyopathy (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 blood

Glycosylated hemoglobin (HbA1C) Assay – HbA1C levels were assayed according to the method of Meyer *et al.*, (1983). Whole blood glycosylated hemoglobin concentration were measured by high performance liquid chromatography (HPLC) using a fully automated Biorad D-10 machine. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the haemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobin then passes through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. In this study Glycosylated hemoglobin (HbA1C) were expressed in percent change.

Parameters assayed in hemolysate

Blood MDA Assay – MDA levels were assayed according to the method of Jain *et al.*, (1989). It is based on thiobarbituric acid (TBA) reactivity. MDA, an end product of fatty acid peroxidation reacts with TBA and form a colored complex that has maximum absorbance at 532 nm. 0.2 ml packed cells or hemolysate was suspended in 0.8 ml phosphate buffered saline and 0.025 ml Butylated hydroxylene. 30% percent TCA was then added. Sample tubes were vortexed and allowed to stand in ice for at least 2 hrs. Sample tubes were centrifuged at 2000 RPM for 15 minutes. One ml of each supernatant was transferred to separate tube and further added 0.075 ml 0.1 mol/L EDTA and 0.25 ml TBA. Sample was mixed and tubes were kept in a boiling water bath for 15 minutes. Absorbance was read at RT at 532 nm and 600 nm. Butylated hydroxylene, an antioxidant was added to prevent MDA formation during the assay, which in order to counter elevated TBA reactivity. The addition of Butylated hydroxylene to standard MDA did not affect the colour development with TBA. Absorbance at 600 nm was subtracted from absorbance at 532 nm. MDA value in nmol/ml packed cells was determined with the absorbance coefficient of MDA – TBA complex at 532 nm = 1.56×10^5 /cm/mol. Plasma or serum MDA levels was measured by reading only at 532 nm. A standard curve was prepared by using 1,1,3 – tetra methoxy propane (TMB). Concentration of MDA is given in nmol/ml/packed cells in hemolysate.

Blood carbonyl protein Assay: Carbonyl protein levels were assayed according to the method of Levine *et al.*, (1990). Equal amount of hemolysate was taken in two different vials. In one vial four volume of DNPH was added in HCl whereas as in second vial four volumes of 2.5 M HCl alone was taken and used as blank. Samples were incubated for one hour at room temperature in the dark, precipitated with 20% and 10% TCA solution, centrifuged and

supernatant was discarded. Washed with equal amount of ethanol & ethyl acetate to remove DNPH and other lipid contaminants. Finally precipitated protein was dissolved in guanidine HCl solution. The absorbance was taken at 375 nm.

Protein Carbonyl (nmol / ml) = Abs 375 x 45.45.

Protein Carbonyl (nmol / mg protein) = $\frac{\text{Protein carbonyl (nmol/ml)}}{\text{Protein concentration (mg/ml)}}$

Protein concentration (mg/ml)

In this study Carbonyl protein were expressed in nmol / mg protein. Serum protein was measured by biuret method (Kingsley.1942).

Superoxide dismutase (SOD): SOD levels were assayed according to the method of Sun.et.al.,(1988). Took equal amount of SOD assay buffer and sample (100 µl) in measuring tube, added 50 µl xanthine solution + 50 µl chromogen or tetrazolium solution + 700 µl distilled water. Finally, added 100 µl of pre-diluted xanthine oxidase solution to each test tube. Mixed well and incubated for one hour at 37⁰C. Read absorbance at 490 nm. SOD activity in hemolysate expressed in U/mg protein or U/g Hb.

Glutathione peroxidase (GPX): GPX levels were assayed according to the method of Pagalia & Valentine.(1967). Spectrophotometer was set zero at 340 nm with deionized water and temperature at 25⁰C before start the test reaction. Mixture of 870 µl working solution, 60 µl start solution, was taken in cuvette, and added 10 µl pre-diluted hemolysate sample. Mixed thoroughly and absorbance was taken at 240 nm after every 15 seconds for 3 minutes. GPX activity in hemolysate expressed in U/gHb.

Glutathione (GSH): GSH levels were assayed according to the method of Beutler.et.al., (1963). Prepared and mix all reagents thoroughly before use. Prepared the glutathione standards simultaneously with the samples so they may be assayed together. Each sample, including unknown and standard, were assayed in duplicate. 25 µL of the 1X glutathione reductase solution and 25 µL of the 1X NADPH solution was mixed with 100 µL of the prepared glutathione standards or samples and mixed thoroughly. Final product produced after adding 50 µL of the 1X chromogen was recorded at the absorbance of 405 nm after every 1 minute intervals for 10 minutes and calculated the concentration of standards and samples. GSH activity in hemolysate expressed in µM.

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 cardiomyopathy was collected from various cardiac centers.

Statistical analysis

The results were expressed as (mean \pm 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. The data management and analyses were performed by using the statistical software SPSS version 11.0.

RESULTS

Glycosylated hemoglobin (HbA1C): Levels of HbA1C in blood samples of poorly controlled diabetic cardiomyopathic subjects were significantly higher ($p < 0.001$) as compared to controlled diabetic cardiomyopathic subjects. Figure.1 shows percent change in poorly controlled type –I diabetic male and female subjects. Increase was 87% & 116% respectively, whereas in type-2 diabetic male subjects increase was 38%, while in females it was 58%. No significant increase in blood HbA1C in controlled type-1 & 2 diabetic cardiomyopathic subjects was observed when compared with non-diabetic subjects.

Malondialdehyde (MDA): MDA levels in blood samples of poorly controlled diabetic cardiomyopathic subjects were found to be significantly higher ($p < 0.001$) as compared to controlled diabetic cardiomyopathic subjects. Figure.2 Shows percent change in type –I diabetic male and female subjects. Increase was 74% & 97% respectively, whereas in type-2 diabetic male subjects increase was 70%, while in type – 2 female diabetic subjects it was 84%. No significant increase in blood MDA in controlled type-1 & 2 diabetic cardiomyopathic subjects was observed when compared with non-diabetic subjects.

Carbonyl content of Protein: Levels of carbonyl protein in blood samples of poorly controlled diabetic cardiomyopathic subjects are significantly higher ($p < 0.001$) as compared to controlled diabetic cardiomyopathic subjects. Figure.3 Shows percent change in type –I diabetic male and female subjects. Increase was 54% & 57% respectively, whereas in type-2

diabetic male subjects increase was 58%, while in type – 2 female diabetic subjects it was 62%. No significant increase in blood carbonyl compounds in controlled type-1 & 2 diabetic cardiomyopathic subjects was observed when compared with non-diabetic subjects.

Superoxide Dismutase (SOD): SOD activity in blood samples of poorly controlled diabetic cardiomyopathic subjects was found to be significantly higher ($p < 0.001$) as compared to controlled diabetic cardiomyopathic subjects. In terms of percent change decrease in SOD activity was 19% in type-1 diabetic male where in 26% in the females. In type-2 diabetic males and females decrease was 33% and 29% respectively (Fig: 4). No significant decrease in blood SOD in controlled type-1 & 2 diabetic cardiomyopathic subjects was observed when compared with non-diabetic subjects.

Glutathione Peroxidase (GPX): GPX activity in blood samples of poorly controlled diabetic cardiomyopathic subjects was found to be significantly higher ($p < 0.001$) as compared to controlled diabetic cardiomyopathic subjects. Figure.5 shows percent change in type –I diabetic male and female subjects. Decrease was -31% & -37% respectively, whereas in type-2 diabetic male subjects in decrease were -28%, while in type – 2 female diabetic subjects it was -31%. Significant decrease in controlled type –I diabetic male and female subject decrease was -25% & -27% respectively, whereas in type-2 diabetic male subjects decrease was -33%, while in type – 2 female diabetic subjects it was -44%.

Total Glutathione (GSH): GSH activity in blood samples of poorly controlled diabetic cardiomyopathic subjects was found to be significantly higher ($p < 0.001$) as compared to controlled diabetic cardiomyopathic subjects. Figure.6 shows percent change in type –I diabetic male and female subjects. Decrease was - 16% & - 20% respectively, whereas in type-2 diabetic male subjects in decrease was - 21%, while in type – 2 female diabetic subjects it was - 29%. No significant decrease in blood GSH in controlled type-1 & 2 diabetic cardiomyopathic subjects was observed when compared with non-diabetic subjects.

Left Ventricular mass (LVM) (ECHO): LVM in ECHO study of poorly controlled diabetic cardiomyopathic subjects was found to be significantly higher ($p < 0.001$) as compared to controlled diabetic cardiomyopathic subjects. Figure.7 shows percent change in type –I diabetic male and female subjects. Increase was 71% & 31% respectively, whereas in type-2 diabetic male subjects increase was 58% and in type – 2 female diabetic subjects it was 33%.

No significant change in LVM in controlled type-1 & 2 diabetic cardiomyopathic subjects was observed when compared with non-diabetic subjects.

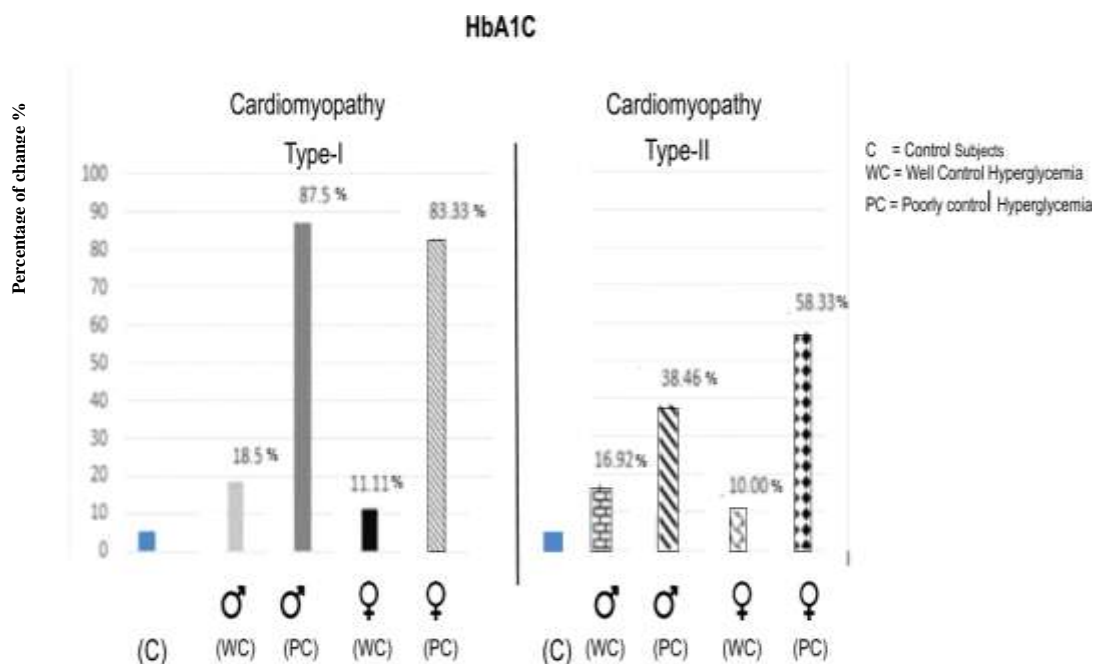


Figure-1 shows the percentage of change in HbA1C of different group with respect to control.

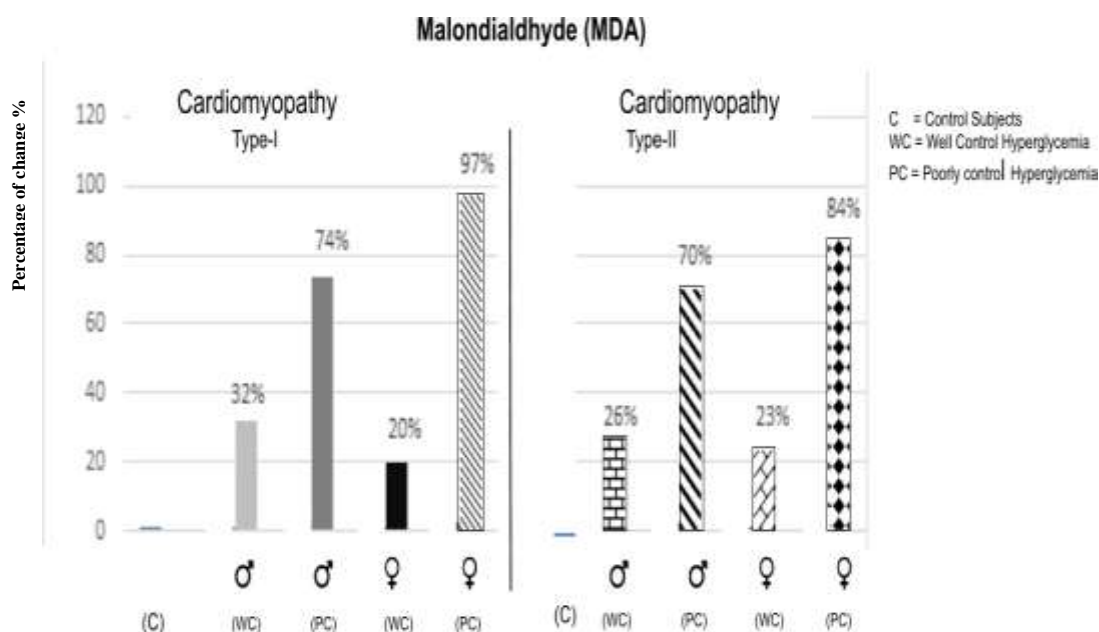


Figure-2 shows the percentage of change in MDA of different group with respect to control.

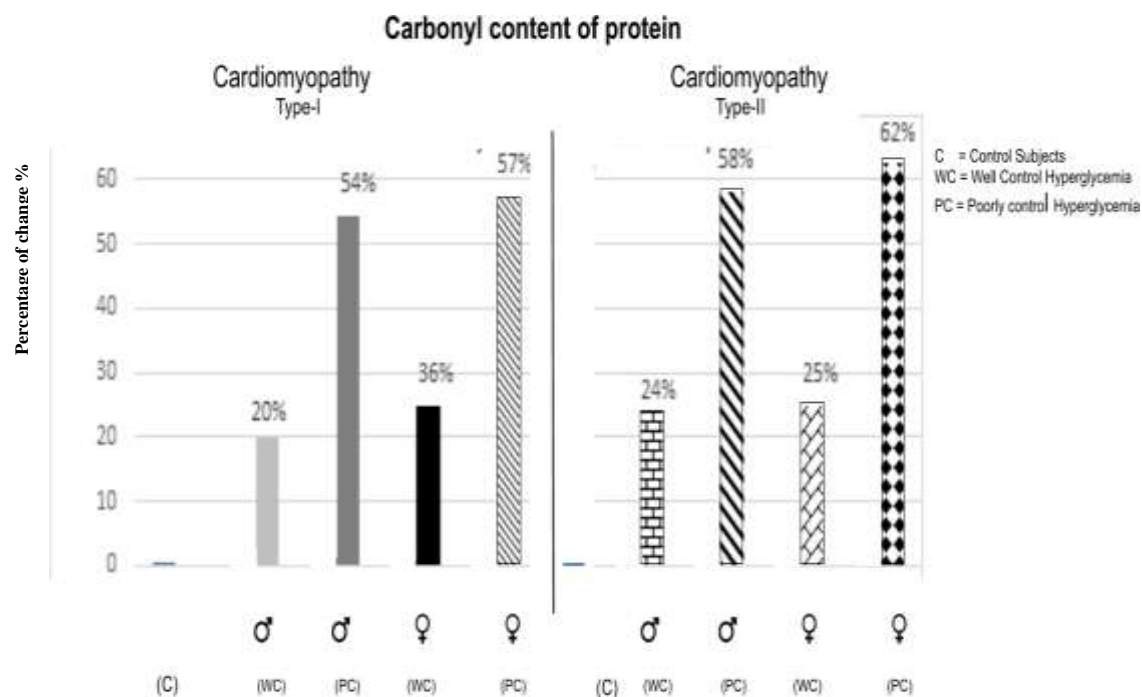


Figure-3 shows the percentage of change in Carbonyl protein of different group with respect to control.

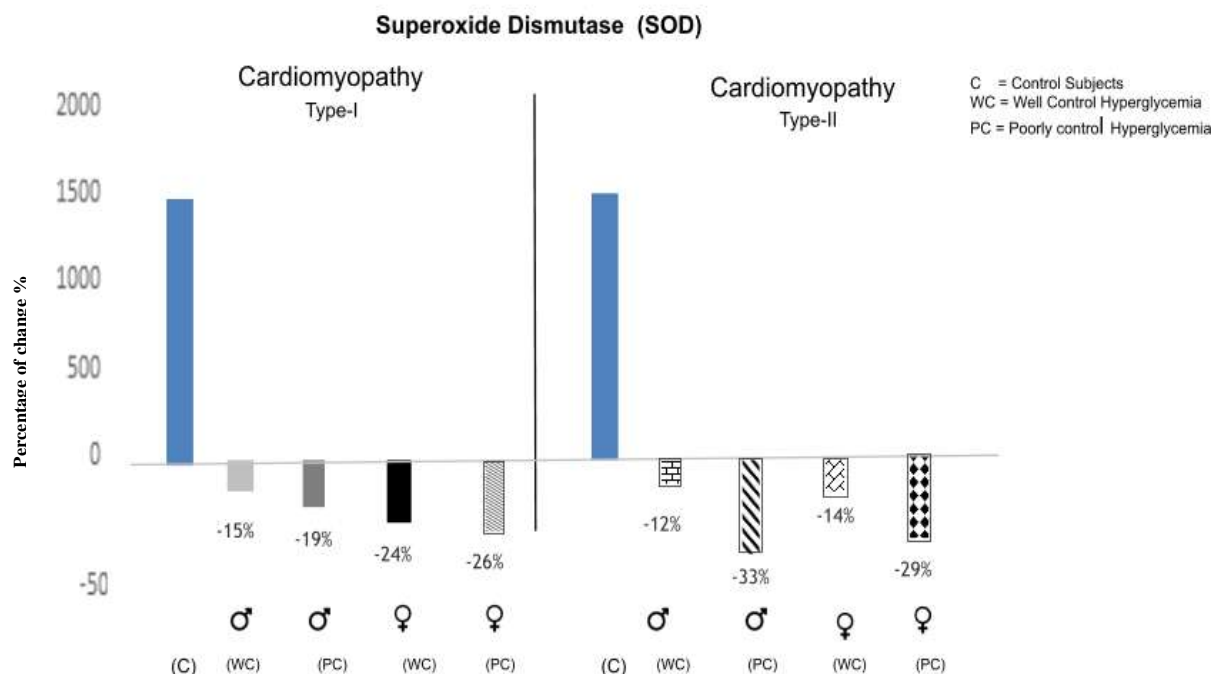


Figure-4 shows the percentage of change in SOD of different group with respect to control.

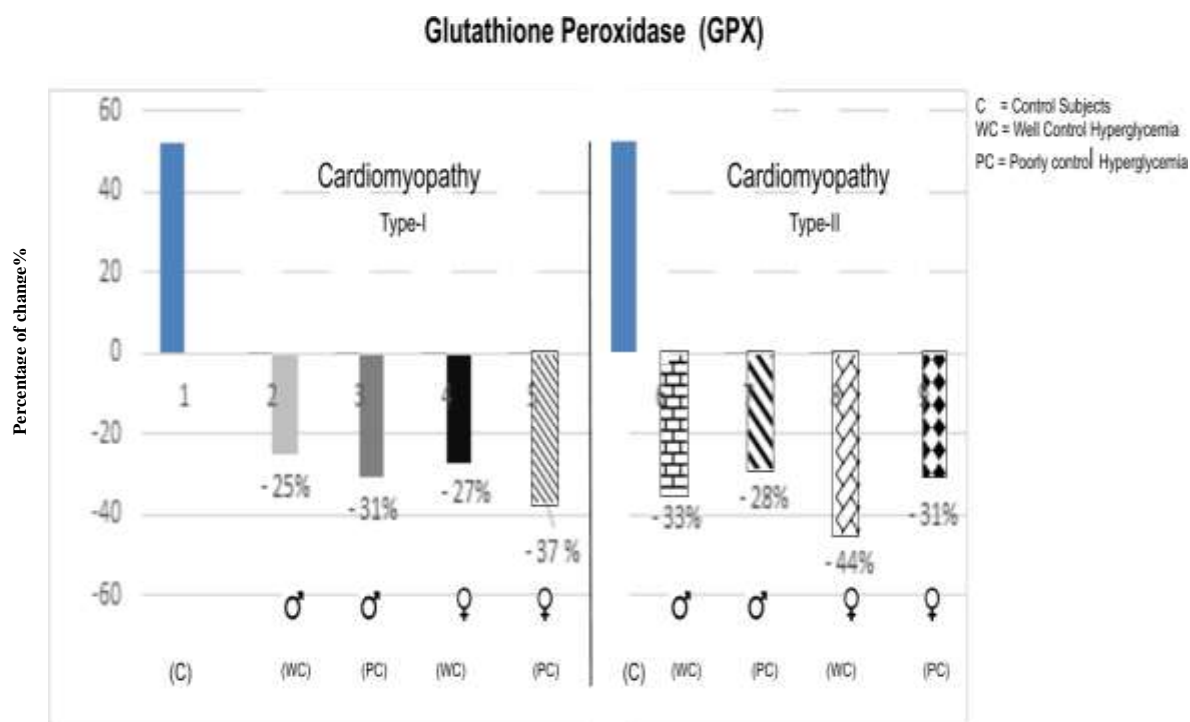


Figure-5 shows the percentage of change in GPX of different group with respect to control.

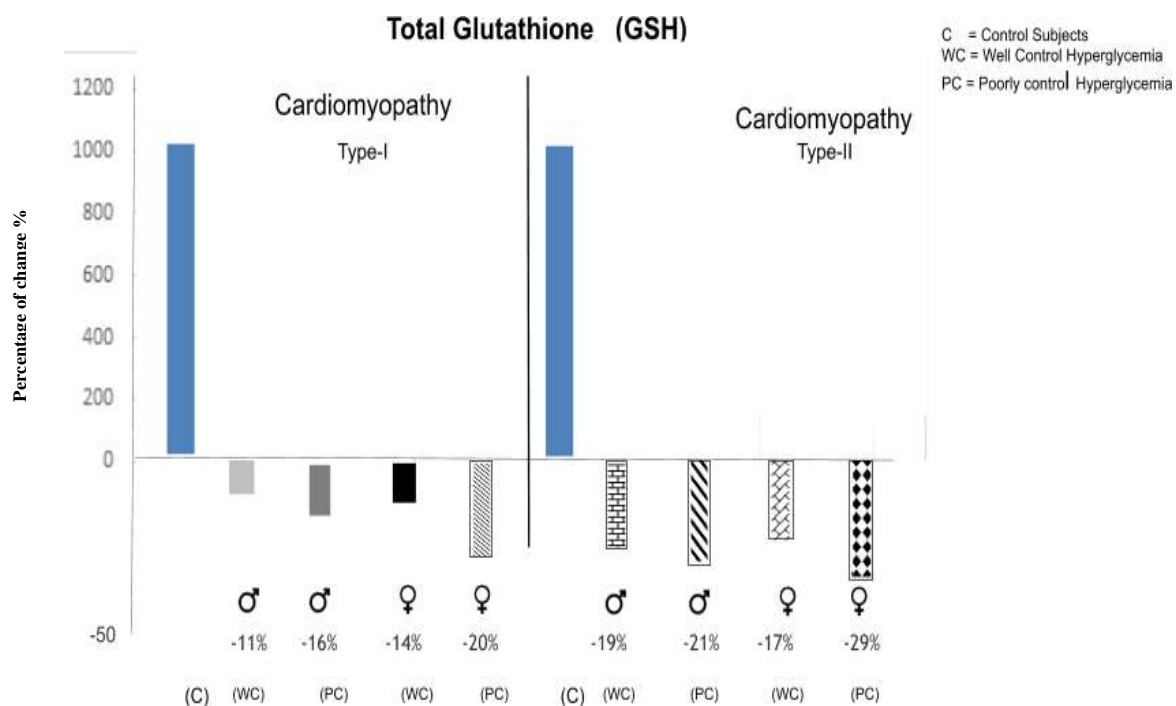


Figure-6 shows the percentage of change in GSH of different group with respect to control.

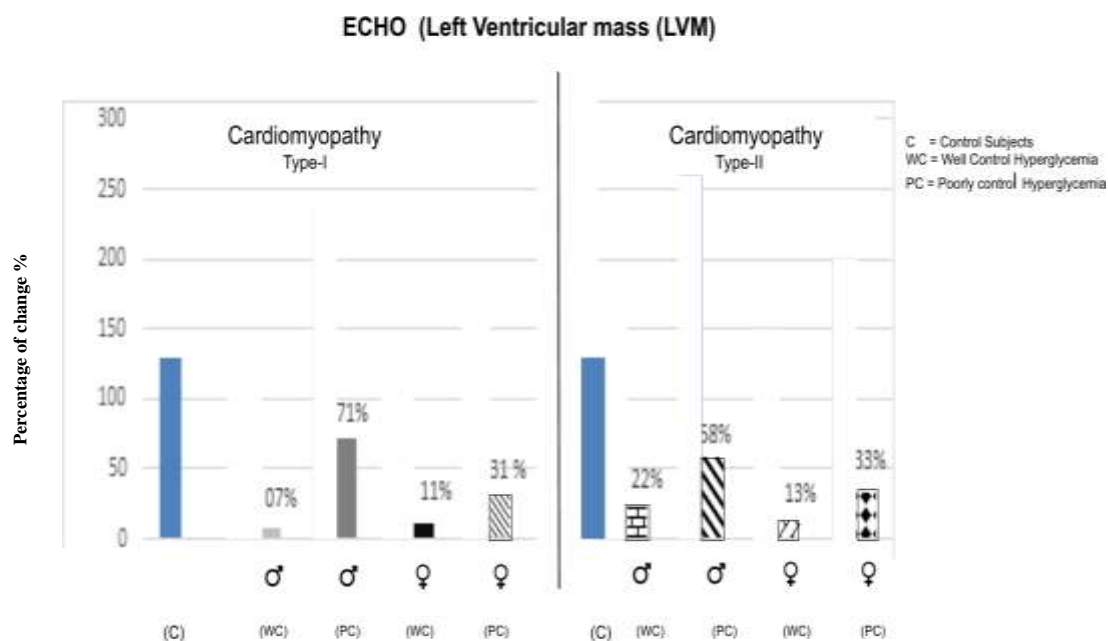


Figure-7 shows the percentage of change in LVM of different group with respect to control.

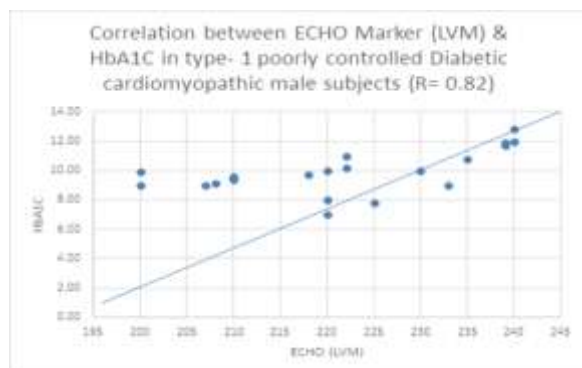


Figure – 8

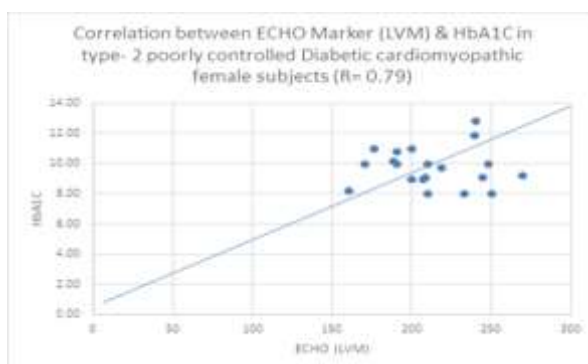


Figure – 9

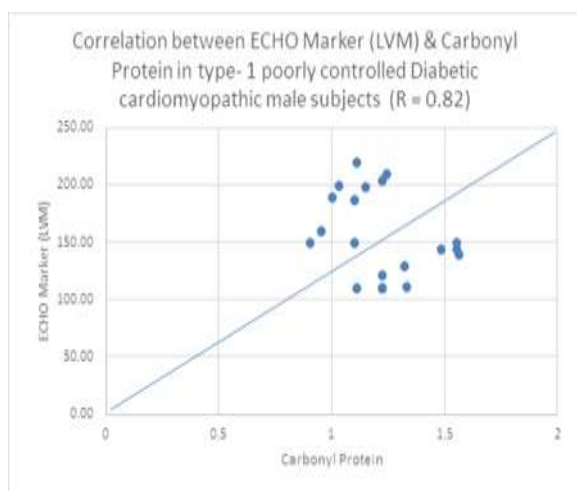


Figure – 10.

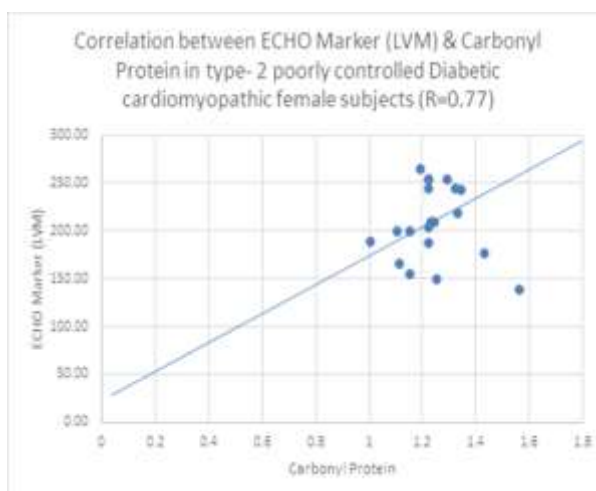


Figure – 11.

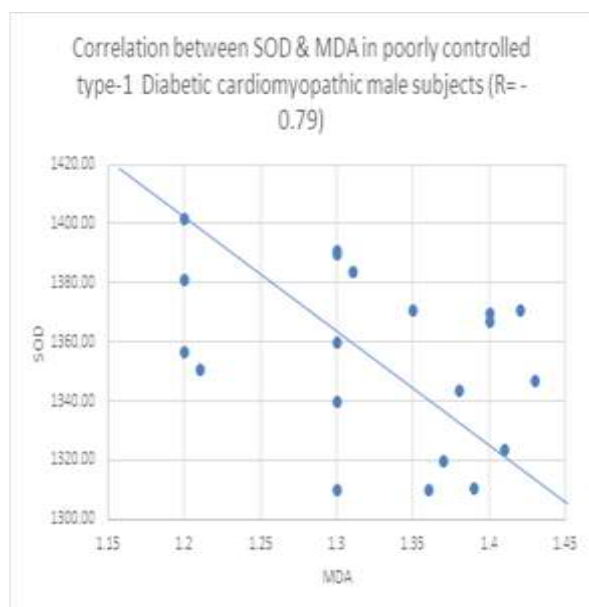


Figure – 12.

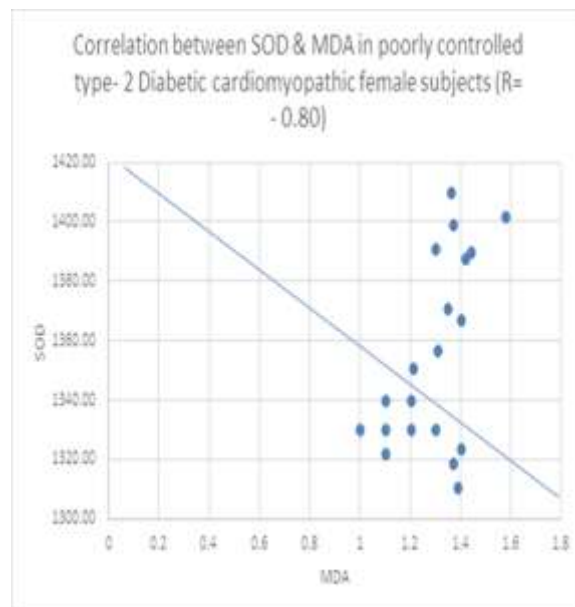


Figure- 13.

Correlation between Hyperglycemia marker (HbA1C) and cardiomyopathic marker (ECHO)

Correlation studies showed a significant positive correlation between HbA1C and ECHO marker in type-1 & type-2 poorly controlled diabetic cardiomyopathic subjects. In Figure – 8 shows type-1 poorly controlled hyperglycemia induced diabetic cardiomyopathic male subjects blood HbA1C and LVM were 12.0 ± 0.8 and 238 ± 15 ($r = 0.82$) and similar correlation found in type-1 poorly controlled diabetic cardiomyopathic female subjects blood HbA1C and LVM were 13.0 ± 0.5 and 190 ± 12 ($r = 0.81$). In Figure – 9 shows type -2 poorly controlled hyperglycemia induced diabetic cardiomyopathic female subjects blood HbA1c and LVM were 9.5 ± 0.8 and 200 ± 19 ($r = 0.79$) and similar correlation found in type-2 poorly controlled hyperglycemia induced diabetic cardiomyopathic male subjects blood HbA1C and LVM were 9.0 ± 1.0 and 264 ± 20 ($r = 0.83$).

Correlation between Oxidative stress marker (Carbonyl compound) and cardiomyopathic marker (ECHO)

Correlation studies showed a significant positive correlation between carbonyl compound and ECHO marker in type-1 & 2 poorly controlled diabetic cardiomyopathic subjects. In Figure-10 shows type-1 poorly controlled hyperglycemia induced diabetic cardiomyopathic (male) subjects blood carbonyl compound level and ECHO (Left ventricular mass (LVM)) were 1.11 ± 0.18 and 238 ± 15 ($r = 0.82$) and similar correlation found in type-1 poorly controlled hyperglycemia induced diabetic cardiomyopathic (female) subjects blood Carbonyl

compound level and ECHO (LVM) were 1.18 ± 0.11 and 190 ± 12 ($r = 0.78$). In Figure-11 shows type – 2 poorly controlled hyperglycemia induced diabetic cardiomyopathic (female) subjects blood carbonyl compound level and ECHO (LVM) were 1.30 ± 0.20 and 200 ± 19 ($r = 0.77$) and similar correlation found in type -2 poorly controlled hyperglycemia induced diabetic cardiomyopathic (male) subjects blood carbonyl compound level and ECHO (LVM) were 1.25 ± 0.15 and 264 ± 20 ($r = 0.79$).

Correlation between Lipid peroxidation (MDA) and Superoxide Dismutase (SOD):

Correlation studies showed a significant negative correlation between lipid peroxidation and SOD activity in male and female type- 1 & 2 diabetic cardiomyopathic subject's blood. In Figure-12 shows type-1 diabetic cardiomyopathic male subjects blood MDA and SOD activity levels were 2.52 ± 0.34 and 1010 ± 55 ($r = - 0.79$) respectively, and similar correlation found in female type – 1 diabetic cardiomyopathic subjects blood MDA and SOD levels were 2.60 ± 0.30 and 882 ± 44 ($r = - 0.77$). In Figure-13 shows in female diabetic cardiomyopathic subjects blood MDA and SOD activity levels were 2.55 ± 0.26 and 880 ± 52 ($r = - 0.80$) respectively and similar correlation found in type – 2 diabetic cardiomyopathic male subjects blood MDA and SOD activity levels were 2.65 ± 0.17 and 910 ± 44 ($r = - 0.78$).

DISCUSSION AND CONCLUSION

There are convincing experimental and clinical evidence that generation of ROS is increased in both types of diabetes mellitus & diabetes induced cardiomyopathy and that the onset of diabetic cardiomyopathy is closely associated with oxidative stress (Moussa.2008). Free radical mediated cytotoxic process of lipid peroxidation appears to have a role in the development of diabetes mellitus and diabetic cardiomyopathy. The increased level of MDA could be because of increased glycation of proteins in diabetes and diabetes induced cardiomyopathy. The glycated protein may themselves act as a source of free radicals. There is a clear association between lipid peroxide and HbA1C which also could be thought to play a role in increased peroxidation in diabetes mellitus (Varashree & Bhat.2011).

Our data shows there was a significant increase in the erythrocyte membrane lipid peroxidation which was shown by increased MDA levels. This increase was seen in both male and female diabetic cardiomyopathic subjects indicating that both the groups are susceptible to diabetes induced oxidative stress. When we compared, we found both type-1 & type-2 diabetic cardiomyopathic female subjects with poorly controlled glycemia have more increased in MDA levels as compared to male subjects. This may be due to auto oxidation of

glucose which causes persistent generation of ROS or MDA, pointing towards the fact that prolonged and poorly controlled hyperglycemia appears to be cause for increased oxidative stress which in turn leads to cardiomyopathy. In poorly controlled hyperglycemia induced cardiomyopathic subjects, we found evidence of systolic and diastolic myocardial dysfunction, especially with the use of ECHO. The correlation between parameters of myocardial function and MDA support a mechanistic role for the increased oxidative stress and myocardial fibrosis in the myocardial dysfunction of type – 2 diabetes (Francisco.et.al., 2004).

Our data showing higher levels of MDA in both male & female subjects with poorly controlled diabetic cardiomyopathy induced heart failure is determined by detailed echocardiographic examination including Left ventricular (LV) diastolic and right ventricular systolic dysfunction is found to be very much consistent with previous study showing increased in MDA levels associated with diabetic heart failure (Shehab.et.al.,2004). The correlation between parameters of myocardial function and MDA support a mechanistic role for the increased oxidative stress and myocardial fibrosis in the myocardial dysfunction of type – 2 diabetes (Ansley & Wang.2013). All these studies directly or indirectly confirming the hypothesis that oxidative stress (increased MDA level) stress is associated to myocardial dysfunction of the heart, which are the signs of diabetic cardiomyopathy.

Data obtained from our study shows tight relationship between levels of blood carbonyl protein, HbA1C and electrophysiological parameters. Increased level of carbonyl or oxidative stress in poorly controlled both male & female diabetic subjects with cardiomyopathy . When we compared, we found both type-1 & type-2 diabetic cardiomyopathic female subjects with poorly controlled glycemia have more increased in carbonyl protein levels as compared to male subjects is reported for the first time in the present study. Survey of literature reveals no specific reason for increased oxidative stress in poorly controlled female diabetic subjects with cardiomyopathy. However, few studies show the pre & postmenopausal, changes in female sex hormone profile & aging could be the reason in female diabetic subjects (Joshi & Aggarwal.2013, Sekhon & Aggarwal.2013). In experimental study on rats after 8 weeks of the induction of diabetes, carbonylation of ventricular myosin heavy chains was reported which demonstrating that heart modification induced by reactive carbonyl species (RCS) may lead to diabetic cardiomyopathy (Shao.et.al.,2012). Carbonylated proteins levels increases in subjects with poorly controlled diabetes mellitus has also been shown by Cakatay.2005.The

present results showing the positive correlation between hyperglycemia induced carbonyl stress and impaired myocardial function of the heart in poorly controlled diabetic subjects are found to be in concurrent with the results of Matough.et.al., 2012 who reported the similar pattern of results in diabetic subjects but with significant rise of the carbonyl content of protein in poorly controlled hyperglycemia induced cardiomyopathic subjects group as compared to controlled hyperglycemia diabetic subjects.

The negative relationship between endogenous scavenger antioxidants (SOD) and levels of HbA1C as well as the positive one both MDA and carbonyl protein products is believed to play important role of glycemic control in antioxidant/oxidant balance in diabetic cardiomyopathic subjects. Our study data reveals that enhanced oxidative stress in diabetic cardiomyopathic female subjects as compared to male subjects was illustrated by the increase in blood lipid peroxidation product (MDA), Carbonyl protein and decrease in antioxidant molecules (SOD). There is variation as to the status of this enzyme in the diabetic and diabetes induced cardiomyopathy state. Some studies have reported decreased SOD activity (Kornatwoska.et.al.,2003 & Obrosova.et.al.,2000) while other have shown increases (Rauscher.et.al.,2001) or no change in the enzyme (Mekinova.et.al.,1995 & Maritim.et.al.,2003). Oxidative stress produce myocardial fibrosis and high left ventricular mass (LVM) is directly related with decreased in blood SOD level. Our data indicate that activity of blood SOD is severely reduced in poorly controlled female diabetic cardiomyopathic subjects as compared to male subjects which is closely related to poor myocardial activity (high Cornell voltage in ECG and increased LVM in ECHO), which is well supported by the recent observation of Doehner.et.al., 2002; Werner.et.al., 2005, who showed that decreased SOD and NO blood levels are associated with poor cardiac outcome. Our study also established a strong link among poor controlled hyperglycemia (HbA1C > 9.0) and oxidative stress and higher LVM in female subjects as compared to male subjects, which is one of ECHO marker for cardiomyopathy. Previous studies show that extracellular superoxide dismutase (SOD) protect the heart from oxidative stress and hypertrophy after myocardial infraction in diabetic subjects (Smith.et.al., 2005). All these studies confirming the hypothesis that decrement in blood SOD level is not only associated with hyperglycemia but also with oxidative stress in diabetic cardiomyopathic subjects.

Data obtained from our study shows slight decrease in the Glutathione Peroxidase (GPX) & GSH level in well controlled diabetic subjects without cardiomyopathy but in poorly

controlled diabetic subjects with cardiomyopathy decrease was significant in both male & female subjects. When we compared, we found both type-1 & type-2 diabetic cardiomyopathic female subjects with poorly controlled glycemia have more decreased in GPX & GSH levels as compared to male subjects. Significant & severe decreased level of blood GPX in female diabetic cardiomyopathic subjects indicates that oxidative stress play a role in cardiac changes or damage. It is known that GPX activity is decreased in patients with diabetes mellitus as well as in experimentally induced diabetic rats (Chiu.et.al.,2005 & dominiguez.et.al.,1998), although some studies have shown opposite results (Maritim.et.al.,2003b). The decrease in GPX activity may contribute to the progression of diabetic complication due to oxidative stress leading to lipid peroxidation and carbonyl stress. Clinical evidence has shown that diabetic patients with cardiomyopathy have significantly lower enzymatic antioxidant defenses, including an impairment in GPX activity, with this defect being more pronounced in younger patients or type – 1 diabetic patients (Colak.et.al.,2005). GPX activity is also found to decrease in diabetic rats in the heart, kidney and brains, leading to enhanced oxidative stress and secondary organ damage (Aliciguzel.et.al.,2003). GSH depletion, increased ROS levels and cardiac apoptosis as early as 4 days after streptozotocin (STZ) administration (Ghosh.et.al.,2004), similar observation were reported when cardiomyocytes were exposed to 24 hrs of high glucose levels in vivo (Fiordialso.et.al.,2004). Cellular glutathione peroxidase (GPX-1) is the intracellular isoform of GPX. GPX-1 deficiency induces endothelial dysfunction and structural abnormalities through increased oxidant stress. Decreased GPX-1 results in impaired endothelium-dependent vascular relaxation in resistance vessels and abnormal diastolic function in hearts subject to diabetes induced ischaemia-reperfusion injury. GPX-1 deficiency causes increased oxidative stress induced vasculature and myocardium of diabetic animals demonstrate increased perivascular matrix deposition and cardiac fibrosis as well as high left ventricular mass (LVM) in ECHO study (Forgione.et.al.,2002). Rutter.et.al.,(2003) demonstrated in diabetic subjects, involved in a Framingham study, that LV mass and wall thickness increased with worsening glucose intolerance and depleted blood antioxidants level, an effects that was more striking in women compared with men. Our data shows a negative correlation between blood GPX & GSH level and electrophysiological parameters (ECG & ECHO) in poorly controlled diabetic subjects with cardiomyopathy, which is in accordance to the above mentioned earlier views. Our data also reveals that levels of GPX and GSH was severely reduced in female subjects as compared to male subjects and strongly correlated with glycemic & ECHO markers. All these studies are in accordance with our data which

shows high glycemic index, and oxidative stress are responsible for the changes in electrophysiological studies of diabetes cardiomyopathy in Indian population.

A comprehensive assessment of cardiac performance by electrophysiological technique made in poorly controlled adult male and female type-1 & type 2 diabetic cardiomyopathy reveals association between diabetes mellitus and abnormal ECHO and ECG findings. Our data indicate that poorly controlled female diabetic subjects with more increased left ventricular mass (LVM) & high cornell voltage as compared to poorly glycemic controlled male subjects. Increased left ventricular mass (LVM) can be seen on female subjects with poorly controlled diabetes, as compared to male subjects with poorly glycemic control suggesting that alteration in the geometry of the heart in diabetic individual are not an early defect but, rather, a consequence of changes associated with diabetes (Rerkpattanapipat.et.al.,2009). Data obtained from our study revealed the positive correlation between oxidative stress and ECG & ECHO study changes in poorly controlled male & female diabetic subjects with cardiomyopathy. In the earlier study on animal model the collagen area in left ventricular myocardial tissue of the STZ rats was shown increased under the influence of oxidative stress. Collagen degradation is largely regulated by Matrix Metalloproteinase (MMP). The decreased MMP-2 gene expression would favor the development of the development of fibrosis (Dai.et.al.,2011, Miric.et.al.,2001, Linthout.et.al.,2008 & Daniels.et.al.,2012). In the diabetic heart, diminished activities of GLUT-4 results in reduced glucose utilization, impaired insulin signaling and increased left ventricular mass (LVM) (How.et.al.,2006). De Marco.et.al., (2011) evaluated echocardiographic data from young diabetic patients differentiating them according to glycemic condition. Their multivariate regression model pointed that poorly controlled hyperglycemia showing left ventricular mass index (LVMI) higher than controls. A study from Velagaleti.et.al.,(2010) demonstrated that pre-diabetic and diabetic individuals showed a direct relationship between their insulin-resistant condition and the ratio between left ventricular mass to end- diastolic volume ratio. Similar type of study by Lee.et.al.,(2011) demonstrated that poorly controlled diabetic individuals showed a direct correlation between QT-interval changes in elctrocardiography (ECG) and left ventricle hypertrophy and increased left ventricular mass (LVM). Various clinical and experimental studies on the role of antioxidants in the reducing the cardiomyopathic changes in diabetic subjects. Dietary supplementation with vitamin-E (2000 IU of tocopherol/kg of feed) beginning early after the onset of type-1 diabetes mellitus in rats and continuing for a period of 8 weeks improved blood antioxidants levels (SOD, GPX, GSH) and significant

improvement in LVSP, LVEDP and reducing left ventricular mass (LVM) compared with unsupplemented DM rats (Hamblin.et.al.,2007b). Oral administration of resveratrol (2.5 mg/kg body wt/day) to STZ- diabetic rats for 15 days has been shown to result in a direct cardioprotective effect on the diabetic myocardium (Thirunavukkarasu.et.al.,2007).Studies have revealed that resveratrol treatment improves cardiac dysfunction of diabetic myocardium in part via modulation of oxidative stress protein (Dekkers.et.al.,2008). All these studies strengthen the hypothesis that poorly controlled hyperglycemia is not only associated with oxidative stress but also responsible for cardiac damages in diabetic cardiomyopathic subjects.

CONCLUSION

Present study made on clinical model, demonstrate poorly controlled diabetes mellitus causes severe cardiomyopathic changes, and we propose that these changes could be mediated by a poorly controlled hyperglycemia induced oxidative stress is found more severe form in female subjects as compared to age matched male subjects.

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