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ORIGINAL DATA

Alpha-Lipoic Acid Improves Subclinical Left Ventricular Dysfunction in Asymptomatic Patients with Type 1 Diabetes

Sahar K. Hegazy[1], Osama A. Tolba[2], Tarek M. Mostafa[1], Manal A. Eid[3], and Dalia R. El-Afify[1]

[1] *Department of Clinical Pharmacy, Faculty of Pharmacy, Tanta University, Egypt.* [2] *Department of Pediatrics, Faculty of Medicine, Tanta University, Egypt.* [3] *Department of Clinical Pathology, Faculty of Medicine, Tanta University, Egypt. Address correspondence to: Dalia R El-Afify, e-mail: daliaelafify@yahoo.com*

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Abstract

BACKGROUND: Oxidative stress plays an important role in the development of diabetic cardiomyopathy. Alpha-lipoic acid (ALA) is a powerful antioxidant that may have a protective role in diabetic cardiac dysfunction. **AIM**: We investigated the possible beneficial effect of alpha-lipoic acid on diabetic left ventricular (LV) dysfunction in children and adolescents with asymptomatic type 1 diabetes (T1D). **SUBJECTS AND METHODS**: Thirty T1D patients (aged 10-14) were randomized to receive insulin treatment (n = 15) or insulin plus alpha-lipoic acid 300 mg twice daily (n = 15) for four months. Age and sex matched healthy controls (n = 15) were also included. Patients were evaluated with conventional 2-dimensional echocardiographic examination (2D), pulsed tissue Doppler (PTD), and 2-dimensional longitudinal strain echocardiography (2DS) before and after therapy. Glutathione, malondialdhyde (MDA), nitric oxide (NO), tumor necrosis factor-alpha (TNF-alpha), Fas ligand (Fas-L), matrix metalloproteinase 2 (MMP-2), and troponin-I were determined and correlated to echocardiographic parameters. **RESULTS**: Diabetic patients had significantly lower levels of glutathione and significantly higher MDA, NO, TNF-alpha, Fas-L, MMP-2, and troponin-I levels than control subjects. The expression of transforming growth factor beta (TGF-beta) mRNA in peripheral blood mononuclear cells was also increased in diabetic patients. Significant correlations of mitral e'/a' ratio and left ventricular global peak systolic strain with glutathione, MDA, NO, TNF-alpha, and Fas-L were observed in diabetic patients. Alpha-lipoic acid significantly increased glutathione level and significantly decreased MDA, NO, TNF-alpha, Fas-L, MMP-2, troponin-I levels, and TGF-beta gene expression. Moreover, alpha-lipoic acid significantly increased mitral e'/a' ratio and left ventricular global peak systolic strain in diabetic patients. **CONCLUSION**: These findings suggest that alpha-lipoic acid may have a role in preventing the development of diabetic cardiomyopathy in type 1 diabetes.

Keywords: type 1 diabetes · diabetic cardiomyopathy · alpha-lipoic acid · oxidative stress · apoptosis · fibrosis · echocardiography

Introduction

Diabetic cardiomyopathy (DCM) is a distinct clinical entity of diabetic heart muscle that describes diabetes-associated changes in the structure and function of the myocardium in the absence of coronary artery disease, hypertension, and valvular disease [1, 2]. The development of DCM is multifactorial and several pathophysiologic mechanisms have been proposed to explain structural and functional changes associated with DCM.

Oxidative stress plays a critical role in DCM development. It has numerous deleterious effects on the cardiovascular system through direct cellular damage of proteins and DNA, activation of apoptosis, and activation of redox transcription nuclear factor κB (NF-κB) which stimulates the

production of inflammatory mediators such as tumor necrosis factor alpha (TNF-α) and interleukin 1β (IL-1β) [3]. These inflammatory mediators can modulate cardiac function, stimulate apoptosis and contribute to the development of DCM [4].

Increased cardiac cell death also plays an important role in the development of DCM. Both apoptosis and necrosis were observed in the hearts of patients with type 1 diabetes (T1D) and type 2 diabetes (T2D) [5]. Hyperglycemia, oxidative stress and inflammation are the main causes of induction of cardiac cell apoptosis in the diabetic heart [6].

The primary structural changes observed in DCM are cardiac fibrosis and accumulation of extracellular matrix proteins, particularly collagen. Collagen accumulation in the diabetic myocardium may be due to either excessive production by fibroblasts or decreased degradation by matrix metalloproteinases (MMPs). Hyperglycemia and oxidative stress cause abnormal gene expression which alters signal transduction, notably activation of NF-κB, which causes upregulation of several genes correlated to fibrosis, such as transforming growth factor-β (TGF-β), in diabetic heart [7].

α-lipoic acid (ALA) has been identified as a powerful antioxidant by its ability to quench reactive oxygen species, decrease oxidative stress, recycle other antioxidants in the body including vitamins C and E and glutathione and protect against protein and lipid oxidation [8, 9]. ALA has been considered to be safe and effective for treatment of symptomatic diabetic polyneuropathy [10, 11]. The aim of the present study was to investigate the possible cardioprotective effect of α-lipoic acid in type 1 diabetic children and adolescents.

Abbreviations:
2D - 2-dimensional echocardiographic examination
2DS - 2-dimensional longitudinal strain echocardiography
a' - peak velocity of mitral annular motion during atrial contraction
A2C - apical 2 chamber
A4C - apical 4 chamber
ALA - alpha-lipoic acid
ALX - apical long axis
AoD - aortic diameter
BDA - BioDocAnalyze
DCM - diabetic cardiomyopathy
DTNB - 5,5'-dithiobis-2-nitrobenzoic acid
e' - early diastolic mitral annular velocity
EF - ejection fraction
ELISA - enzyme-linked immunosorbent assay
eNOS - endothelial NOS
Fas-L - Fas ligand
FBG - fasting blood glucose
fps - frames per second
GPSS - global peak systolic strain
HbA1c - glycosylated hemoglobin
IL-1beta - interleukin 1beta
iNOS - inducible NOS
LAD - left atrial diameter
LV - left ventricular
LVIDd - left ventricular internal diastolic diameter
MDA - malondialdhyde
MMP - matrix metalloproteinase
NF-κB - nuclear factor κB
NO - nitric oxide
NOS - nitric oxide synthase
nNOS - neuronal NOS
OD - optical density
PBMC - peripheral blood mononuclear cell
PSS - peak systolic strain
PTD - pulsed tissue Doppler
RNA - ribonucleic acid
RT-PCR - reverse transcription polymerase chain reaction
s - peak mitral annulus systolic velocity
SD - standard deviation
SPSS - Statistical Package for Social Science
T1D - type 1 diabetes
T2D - type 2 diabetes
TBARS - thiobarbituric acid reactive substances
TGF-beta - transforming growth factor beta
TNF-alpha - tumor necrosis factor alpha

Subjects and methods

Study population

Thirty children and adolescents with T1D recruited from the Diabetic Outpatients Clinic of the Endocrinology Unit, Pediatric Department, Tanta University Hospitals and 15 healthy controls of matched age and sex were included in the study. Inclusion criteria were 10 years or more of age, one year or more of diabetes duration and no clinical evidence of heart disease. Exclusion criteria were clinical evidence of heart failure, coronary artery disease, systemic hypertension, rheumatic fever, cardiomyopathy, and the use of any medication other than insulin known to affect cardiac function (such as digitalis, angiotensin converting enzyme inhibitor, or β-blocker). The study was approved by the Research Ethics Committee of the Faculty of Medicine, Tanta University. Written consents were obtained from parents of all participants. Patients were randomized to group A which received insulin alone (n = 15) or group B which received insulin plus ALA 300 mg twice daily (n = 15) for four months.

Blood samples, obtained after at least 8 hours fasting, were taken at baseline for all participants and after the end of a four-month treatment period in patient groups to measure several biochemical markers.

Table 1. Nucleotide sequence for RT-PCR

Primer	Sequence	Product size
β-actin	F: 5' GTG GGG CGC CCC AGG CAC CA 3'	497 bp
	R: 5' GTC CTT AAT GTC ACG CAC GAT TTC 3'	
TGF-β	F: 5' ATC AGA GCT CCG AGA AGC GGT ACC 3'	280 bp
	R: 5' GTC CAC TTG CAG TGT GTT ATC CCT G 3'	

Biochemical measurements

Fasting blood glucose (FBG) and serum total cholesterol were determined using commercially available reagent kits (Spinreact, Ctra. Santa Coloma, Spain and ELITECH diagnostics, Seppim SA, France respectively). Hemoglobin A1c (HbA1c) was measured by an ion exchange chromatographic spectrometric method using a commercially available kit (Biosystems reagents, Ctra. Santa Coloma, Spain).

Determination of glutathione, malondialdhyde and nitric oxide

Glutathione was determined in total blood using the method described by Chavan *et al.* [12]. This method is based on reductive cleavage of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) reagent by the sulfhydryl group of reduced glutathione to yield a yellow color, measured at 412 nm.

Plasma malondialdhyde (MDA) was estimated by determination of thiobarbituric acid reactive substances (TBARS) using the method of Draper and Hadly [13]. The method depends on the reaction between MDA and thiobarbituric acid in an acidic medium at high temperature to produce a pink color product, which is extracted in n-butanol and measured at 535 nm.

Plasma nitric oxide (NO) was determined by measuring total nitric oxide metabolites (nitrate plus nitrite), using the method developed by Miranda *et al.* [14]. This method depends on the reduction of nitrate to nitrite using vanadium (III), followed by the addition of Griess reagents which produce a colored product, measured at 540 nm.

Determination of TNF-alpha, Fas-L, MMP-2, and troponin-I

Serum concentration of TNF-α, Fas-L, MMP-2, and troponin-I were measured using commercially available ELISA assay kits (Orgenium Laboratories, Vantaa, Finland; RayBiotech Inc., Norcross, USA; SunRed Biotech, Shanghai, PRC and Monobind Inc., Lake Forest, USA respectively).

Semiquantitative analysis of TGF-beta mRNA level in peripheral blood mononuclear cells (PBMCs) using RT-PCR

Peripheral blood mononuclear cells were isolated using the Ficoll-Paque density-gradient centrifugation technique. Total RNA was extracted from PBMCs using the RNA Purification Mini Kit (Thermo Fisher Scientific Inc., California, USA) as described by the manufacturer. RT-PCR was carried out using the 1-Step RT-PCR Kit (Thermo Fisher Scientific Inc.). The housekeeping β-actin was simultaneously amplified with each sample. The sequence of the primers is listed in **Table 1**. The following cycle conditions were applied: initial cDNA synthesis at 50ºC for 15 min followed by denaturation at 95ºC for 2 min and amplification by 40 cycles consisting of denaturation at 95ºC for 20 s, annealing at 55ºC for 30 s, and extension at 72ºC for 1 min, followed by a final 10 min extension at 72ºC. The amplified RT-PCR products were visualized on a 2% agarose gel with ethidium bromide

Table 2. Clinical data of diabetic patients and controls

Parameter	Control	Patients	
		Group A (n = 15)	Group B (n = 15)
Age (yr)	11.5 ± 1.4	11.1 ± 2.3	11.9 ± 1.4
Gender (m/f)	7/8	7/8	7/8
Weight (kg)	39.3 ± 6.8	35.0 ± 8.6	41.4 ± 7.6
Height (kg)	138.0 ± 12.5	131.4 ± 16.0	143.0 ± 13.9
BMI (kg/m^2)	20.6 ± 1.8	20.0 ± 1.3	20.2 ± 1.3
Duration of diabetes (yr)	-	4.3 ± 2.1	4.4 ± 3.0

Legend: Data are mean ± SD or number. Group A: diabetic patients given insulin alone. Group B: diabetic patients given insulin plus ALA 300 mg twice daily. BMI: body mass index.

Table 3. Biochemical data of patient groups and controls before and after drug treatment

Parameter	Control	Group A (n = 15)		Group B (n = 15)	
		Before treatm.	After treatm.	Before treatm.	After treatm.
FBG (mg/dl)	83.0 ± 6.5	168.0 ± 26.4[a]	162.0 ± 25.6	166.0 ± 30.0[a]	161.0 ± 32.2
Cholesterol (mg/dl)	144.0 ± 7.8	149.0 ± 12.8	148.0 ± 10.8	147.0 ± 13.8	146.0 ± 12.3
HbA1c (%)	5.4 ± 0.4	9.2 ± 1.7[a]	9.3 ± 1.6	10.2 ± 1.6[a]	10.1 ± 1.5
Glutathione (mg/dl)	42.8 ± 7.3	26.3 ± 5.4[a]	24.9 ± 7.5	24.1 ± 6.2[a]	32.1 ± 7.0[b,c]
MDA (nmol/ml)	2.2 ± 0.9	4.1 ± 1.1[a]	4.0 ± 0.9	4.0 ± 1.0[a]	3.2 ± 0.8[b,c]
Nitric oxide (µmol/l)	34.0 ± 5.5	45.6 ± 5.7[a]	45.8 ± 5.7	46.8 ± 5.4[a]	35.1 ± 6.0[b,c]
TNF-α (pg/ml)	5.4 ± 1.7	9.0 ± 0.9[a]	9.1 ± 1.0	9.1 ± 0.9[a]	7.2 ± 0.7[b,c]
Fas-L (pg/ml)	85.0 ± 6.5	124.0 ± 16.0[a]	125.0 ± 15.7	126.0 ± 12.4[a]	99.0 ± 13.7[b,c]
MMP-2 (ng/ml)	435.0 ± 171.0	681.0 ± 176.0[a]	670.0 ± 157.0	667.0 ± 185.0[a]	536.0 ± 157.0[b,c]
Troponin-I (ng/ml)	< 0.01	0.031 ± 0.005[a]	0.032 ± 0.005	0.032 ± 0.003[a]	0.025 ± 0.003[b,c]

Legend: Data are means ± SD. Group A: diabetic patients given insulin alone. Group B: diabetic patients given insulin plus ALA 300 mg twice daily. [a] Significant different compared with controls ($p < 0.05$). [b] Significant different after treatment compared with their respective values before treatment ($p < 0.05$). [c] Significant different compared with group A after treatment ($p < 0.05$).

and quantified using BioDocAnalyze (BDA) Software.

Echocardiography

Echocardiographic images were obtained using a Vivid 7 ultrasound machine (GE Medical System, Horten, Norway with a 3.5-MHz multifrequency transducer). All measurements were carried out by the same echocardiographer, who was blinded to the treatment arm to which subjects belonged, at the same time of day to avoid possible bias. Conventional 2-dimensional echocardiographic examination (2D) was performed including aortic diameter (AoD), left atrial diameter (LAD), left ventricular internal diastolic diameter (LVIDd) and ejection fraction (EF). Pulsed tissue Doppler (PTD) was carried out with a sample volume of 5.5 mm and frame rate greater than 150 fps. 3 consecutive cycles were recorded. The parameters measured with PTD were early diastolic mitral annular velocity (e'), peak velocity of mitral annular motion during atrial contraction (a'), e'/a' ratio, and mitral annulus systolic velocities (s). 2-dimensional longitudinal strain (2DS) echocardiogram images were obtained using the 3 standard apical views; apical long axis (ALX), apical 4 chamber (A4C), and apical 2 chamber (A2C) views. The parameters obtained represent the average of 3 cardiac cycles, with a frame rate of 65 fps. We used automated function imaging which enables the assessment of longitudinal strain and is available in the Vivid 7 ultrasound machine to measure average left ventricular global peak systolic strain and peak systolic strain in 3 standard apical views.

Statistical analysis

Data were analyzed using SPSS software version 17 and presented as mean ± standard deviation (SD). The differences between the results obtained in the groups studied before drug administration were assessed by the Kruskal-Wallis test followed by the Wilcoxon-Mann-Whitney test. The Wilcoxon signed-rank test was also used to assess any significant difference within each patient group before and after drug treatment. The differences between group A and B after drug treatment were evaluated using the Wilcoxon-Mann-Whitney test. Correlation between biochemical and echocardiographic parameters was evaluated using Spearman's rank correlation coefficient. A p-value < 0.05 was considered statistically significant.

Results

The participants' clinical data are presented in **Table 2.** The control and patient groups did not significantly differ in relation to age, weight, height, and body mass index. The biochemical results for control and patients before and after drug treatment are listed in **Table 3**.

T1D patients from both group A and B included in our study had normal total cholesterol levels when compared with healthy controls and the total

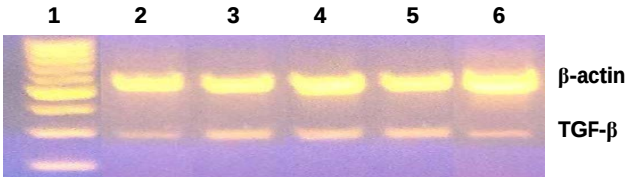

Figure 1. Representative agarose gel electrophoresis profiles of TGF-β mRNA amplification stained with ethidium bromide where: (1) DNA Marker, (2) control, (3, 4) diabetic patients in group A before and after treatment, (5, 6) diabetic patient in group B before and after ALA treatment.

cholesterol levels were not significantly changed after the administration of either insulin alone in group A or ALA in group B. FBG and HbA1c were significantly higher in both group A and B compared to healthy control groups before the drug therapy. At the end of the four-month treatment period, neither FBG nor HbA1c was significantly changed in either group A or B. Moreover, there were no significant differences in FBG and HbA1c between group A or B after drug treatment.

Before drug administration, both group A and B had significantly lower glutathione and significantly higher MDA and NO levels compared to controls. Glutathione, MDA, and NO levels were not significantly changed in group A after administration of insulin alone for four months. On the other hand, group B showed a significant increase in glutathione level by 33% and a significant decrease in MDA level by 20% and NO level by 25% after four months' administration of ALA compared with baseline values before drug treatment. In addition, glutathione level was significantly higher and MDA and NO levels were significantly lower in group B compared with group A after four months' administration of drug therapy.

Levels of TNF-α, Fas-L, MMP-2, and troponin-I were significantly higher in both group A and B compared to controls, before drug administration. Group A did not show any significant change in TNF-α, Fas-L, MMP-2, and troponin-I level after administration of insulin alone for four months. However, group B showed a significant decrease in TNF-α level and Fas-L level by 21%, MMP-2 by 20%, and troponin-I level by 22% after four months' administration of ALA compared with their baseline values before drug treatment. Moreover, TNF-α, Fas-L, MMP-2 and troponin-I levels were significantly lower in group B compared with group A after four months' administration of drug therapy.

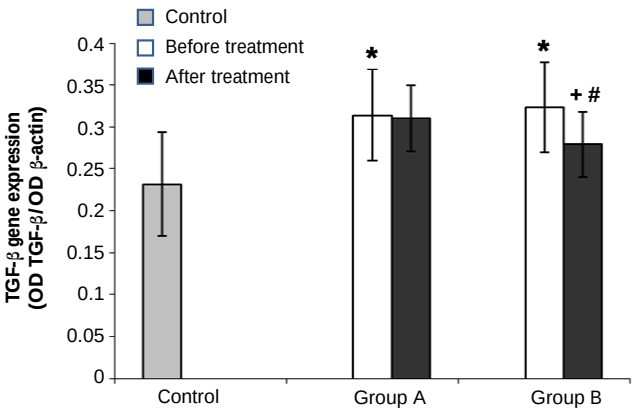

Figure 2. Ratio of the optical density (OD) of samples to the corresponding housekeeping gene (β-actin). Each point represents the mean ± SD. * Indicates a significant difference compared with the control group ($p < 0.05$). + Indicates a significant difference after treatment compared with their respective values before treatment ($p < 0.05$). # indicates a significant difference compared with group A after treatment ($p < 0.05$). Group A: diabetic patients given insulin alone. Group B: diabetic patients given insulin plus ALA 300 mg twice daily.

Gene expression of TGF-beta in PBMCs

Representative agarose gel electrophoresis profiles of TGF-β mRNA amplification stained with ethidium bromide for controls and patients before and after treatment are shown in **Figure 1**. TGF-β gene expression was significantly increased in diabetic groups compared with controls before drug treatment. A significant reduction in TGF-β gene expression level was observed after administration of ALA compared with before treatment or with group A as shown in **Figure 2**.

Echocardiography

The echocardiographic results are listed in **Table 4**. No significant differences in AoD, LAD, LVIDd, EF, peak mitral annulus systolic velocity (s), diastolic mitral annular velocity (e'), and peak velocity of mitral annular motion during atrial contraction (a') were found between controls and both group A and B before the drug administration. The previous parameters were not significantly changed after the administration of either insulin alone in group A or ALA in group B. However, the mitral e'/a' ratio was significantly lower in diabetic patients in both group A and B com-

Table 4. Echocardiographic data of patient groups and controls before and after drug treatment

Parameter	Control	Group A (n = 15)		Group B (n = 15)	
		Before treatm.	After treatm.	Before treatm.	After treatm.
AoD (cm)	2.13 ± 0.30	2.23 ± 0.22	2.24 ± 0.24	2.17 ± 0.24	2.16 ± 0.23
LAD (cm)	2.83 ± 0.34	2.81 ± 0.25	2.82 ± 0.26	2.89 ± 0.35	2.90 ± 0.32
LVIDd (cm)	4.49 ± 0.27	4.57 ± 0.18	4.59 ± 0.16	4.36 ± 0.38	4.33 ± 0.34
EF (%)	69.20 ± 5.50	68.70 ± 6.70	69.00 ± 6.80	68.80 ± 6.20	69.40 ± 6.10
Mitral s (m/sec)	0.07 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Mitral e' (m/sec)	0.12 ± 0.03	0.12 ± 0.03	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.02
Mitral a' (m/sec)	0.07 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.07 ± 0.02
Mitral e'/a' (m/sec)	1.88 ± 0.49	1.47 ± 0.35[a]	1.41 ± 0.34	1.49 ± 0.28[a]	1.69 ± 0.35[b,c]
PSS ALX (%)	-19.90 ± 3.15	-17.51 ± 5.50	-16.79 ± 4.90	-16.93 ± 3.30	-19.13 ± 5.83
PSS A4C (%)	-20.20 ± 3.42	-17.34 ± 4.60[a]	-17.02 ± 3.76	-15.26 ± 3.06[a]	-21.26 ± 3.80[b,c]
PSS A2C (%)	-19.90 ± 2.12	-15.31 ± 4.75[a]	-14.03 ± 3.02	-13.62 ± 2.66[a]	-18.52 ± 3.84[b]
LV GPSS (%)	-21.60 ± 1.64	-16.69 ± 4.63[a]	-14.90 ± 3.96	-15.27 ± 3.51[a]	-20.89 ± 3.53[b,c]

Legend: Data are means ± SD. Group A: diabetic patients given insulin alone. Group B: diabetic patients given insulin plus ALA 300 mg twice daily. [a] Significant different compared with controls ($p < 0.05$). [b] Significant different after treatment compared with their respective values before treatment ($p < 0.05$). [c] Significant different compared with group A after treatment ($p < 0.05$). *Abbreviations*: AoD – aortic dimension, LAD – left atrial dimension, LVIDd – left ventricular internal diastolic dimension, EF – ejection fraction, s – tissue Doppler peak mitral annulus systolic velocity, e' – early diastolic mitral annular velocity, a' – peak velocity of mitral annular motion during atrial contraction, PSS ALX – peak systolic strain in apical long axis view, PSS A4C – peak systolic strain in apical 4 chamber view, PSS A2C – peak systolic strain in apical 2 chamber view, LV GPSS – left ventricular global peak systolic strain.

pared to healthy controls before drug administration. The mitral e'/a' ratio was not significantly changed in group A after administration of insulin alone for four months. In contrast, the mitral e'/a' ratio was significantly increased by 13% in group B after administration of ALA for four months compared with their baseline values before drug treatment. In addition, the mitral e'/a' ratio was significantly higher in group B than in group A after four months' administration of drug therapy.

In 2-dimensional longitudinal strain, the 3 standard apical views showed that group A as well as group B had significantly lower peak systolic strain (PSS) in the A4C and A2C views and significantly lower left ventricular global peak systolic strain (LV GPSS) compared to controls before drug therapy. Group A did not show any significant change in PSS A4C, PSS A2C and LV GPSS at the end of four months' administration of insulin alone. However, a significant increase occurred in PSS A4C by 39%, PSS A2C by 36% and LV GPSS by 37% in group B after four months' administration of ALA compared with their baseline values before drug treatment. Moreover, PSS A4C and LV GPSS were significantly higher in group B compared with group A after four months' administration of drug therapy.

Correlation between biochemical and echocardiographic parameters was evaluated using Spearman's rank correlation coefficient, and $p < 0.05$ was considered statistically significant. There were significant negative correlations between LV GPSS and glutathione ($r = -0.652$), and significant positive correlations between LV GPSS and MDA ($r = 0.49$), NO ($r = 0.485$), TNF-α ($r = 0.373$), and Fas-L ($r = 0.585$) in diabetic patients. Furthermore, a significant positive correlation between e'/a' ratio and glutathione ($r = 0.588$), significant negative correlations between e'/a' and MDA ($r = -0.481$), NO ($r = -0.453$) and TNF-α ($r = -0.403$) and Fas-L ($r = -0.378$) were also observed. However, neither LV GPSS nor e'/a' had significant correlation with MMP-2 ($r = -0.063$ and -0.164 respectively).

Troponin-I showed significant negative correlations with glutathione ($r = -0.418$) and significant positive correlations with MDA ($r = 0.397$), NO ($r = 0.504$), and Fas-L ($r = 0.397$). However, it had no significant correlation with TNF-α, MMP-2 ($r = -0.067$ and 0.187 respectively), e'/a' ratio, and LV

GPSS in diabetic patients (r = -0.09 and 0.175 respectively).

Discussion

The natural history of DCM consists of a latent subclinical period, during which cellular structural insults and abnormalities occur initially leading to diastolic dysfunction and progressing to degenerative changes, which the myocardium is unable to repair, with subsequent irreversible pathological remodeling [15]. Recent echocardiographic modalities (tissue Doppler and 2-dimensional longitudinal strain) represent a diagnostic method that can help in early detection of DCM and can evaluate diastolic and systolic heart dysfunction.

Pulsed tissue Doppler showed that type 1 diabetic patients had abnormal diastolic function manifested as significantly lower mitral e'/a' ratio. However, 2-dimensional longitudinal strain showed that the patients had abnormal systolic function presented by significantly lower LV global peak systolic strain compared to that of controls. These results are consistent with other studies which have demonstrated that tissue Doppler and 2-dimensional longitudinal strain have the potential for detecting subclinical diastolic and systolic dysfunction in the asymptomatic diabetic population [16-18].

On the other hand, conventional echocardiography was unable to detect left ventricular systolic or diastolic dysfunction in diabetic patients because the early stages of DCM do not cause any changes in myocardial structure and architecture; therefore the internal dimensions of cardiac cavities were normal. However, the lesions associated with the early stages of DCM occur at a myocytic level, are functionally expressed, and can be detected only with recent echocardiographic techniques.

Glutathione is the most abundant intracellular antioxidant in all cells while MDA is the product of polyunsaturated fatty acid peroxidation. Measurement of glutathione and MDA indirectly reflect the degree of oxidative stress. Diabetic patients had significantly low glutathione and high MDA, an increase in oxidative stress that has also been reported by others [19, 20]. The significant correlations of serum levels of glutathione, MDA, and NO with e'/a' ratio and ventricular global peak systolic strain in diabetic patients is a mirror image of the key role of oxidative stress in the pathogenesis of DCM.

ALA increased glutathione and decreased MDA, which can be explained by the ability of ALA to regenerate glutathione [9]. Furthermore, ALA has been reported to increase glutathione synthesis by increasing cellular uptake of the cysteine required for glutathione synthesis [21]. The decrease in MDA levels can be explained by the antioxidant ability of ALA and its ability to regenerate and to increase glutathione levels. These results are in agreement with Borcea *et al.* who demonstrated that ALA significantly improves antioxidant defense and decreases oxidative stress in diabetic patients, even in patients with poor glycemic control [22].

Nitric oxide is an important regulator of cardiac function which is synthesized by three distinct isoforms of nitric oxide synthase (NOS) within the myocardium. Neuronal NOS (nNOS) and endothelial NOS (eNOS) produce NO to modulate cardiac function. On the other hand, inducible NOS (iNOS) produces high levels of NO and is only expressed during the inflammatory response of many pathophysiological conditions of the myocardium (ischemia-reperfusion injury, septicemia, heart failure, etc.) mediating a decrease in cardiac myocyte contraction, inducing apoptosis, and leading to the formation of the strong oxidant peroxynitrite [23].

Hyperglycemia and oxidative stress increase the expression of iNOS through the activation of NF-κB [24] and protein kinase C [25]. The increased expression of iNOS may explain the increase in plasma NO concentration in diabetic patients which was also observed in previous studies [26, 27]. ALA decreased NO, probably because of its ability to reduce oxidative stress-mediated NF-κB activation and subsequently iNOS expression in diabetic patients [28-30].

Hyperglycemia, oxidative stress and activation of the renin-angiotensin system induce inflammatory responses which contribute to the development of DCM [4, 31]. Cardiac inflammation in DCM, as well as heart failure, is accompanied by elevated cardiac cytokines levels including TNF-α, IL1-β, IL-6, and TGF-β [4].

TNF-α is one of the main pro-inflammatory cytokines involved in DCM. It can contribute to cardiac failure by stimulating myocyte hypertrophy, myocardial fibrosis [4], and apoptosis [6]. The high level of TNF-α observed in diabetic patients is compatible with that reported in other previous studies [32, 33]. The significant correlation of TNF-α with e'/a' ratio and ventricular global peak systolic strain in diabetic patients may reflect the role of inflammatory cytokines in the pathogenesis of DCM.

TGF-β is a profibrotic cytokine that stimulates the production of extracellular matrix proteins in different organs. In the heart, TGF-β induces the differentiation of cardiac fibroblasts to the more active myofibroblasts, which can produce up to two-fold more collagen than their fibroblast precursors [34]. The increased expression of TGF-β in our diabetic patients is consistent with animal studies that showed upregulation of TGF-β mRNA in the hearts of diabetic animals [7, 35].

Hyperglycemia and oxidative stress activate NF-κB, which regulates the expression of large numbers of genes including pro-inflammatory cytokines (TNF-α and IL-1β) and several genes correlated to fibrosis, including TGF-β, in the diabetic heart [7, 36]. ALA can scavenge intracellular free radicals and therefore down-regulate pro-inflammatory redox-sensitive signal transduction processes including NF-κB activation [28, 29]. The decrease in TNF-α levels and TGF-β expression in patients who received ALA in our study can be explained by the ability of α-lipoic acid to suppress NF-κB activation.

Oxidative stress is the critical and central mediator involved in diabetes-induced myocardial cell death [6]. Oxidative stress can activate the cytochrome C-activated caspase-3 and the death receptor pathways [37, 38]. Activated TNF and the Fas/Fas ligand system play a significant role in the apoptosis of cardiomyocytes [39] and this may explain high Fas-L levels in diabetic patients. In addition, elevated levels of circulating Fas-L was found in heart failure patients and was related to myocardial damage [40]. The significant correlations of Fas-L and TNF-α with e'/a' ratio and ventricular global peak systolic strain in diabetic patients may demonstrate that apoptosis plays a role in the pathogenesis of DCM. The ability of ALA to lower Fas-L level in our study is consistent with Bojunga *et al.* who reported that ALA decreased Fas-L gene expression in the hearts of diabetic animals and prevented the activation of death receptor signaling [41].

The increased serum MMP-2 concentration in diabetic patients is contradictory with the results of studies that revealed decreased expression and activity of MMP-2 in cardiac tissue of diabetic animals [42, 43]. It has been reported that hyperglycemia induces upregulation of MMP-2 in human arterial vasculature via oxidative stress and advanced glycation end-products [44]. Therefore, the increase in MMP-2 could be due to its increased vascular synthesis or could reflect the systemic transport of MMP-2, which is being overproduced in tissues other than the myocardium. This may also explain the lack of significant correlations of MMP-2 with the e'/a' ratio, LV global peak systolic strain, and troponin-I in diabetic patients. The decrease of MMP-2 by α-lipoic acid may be explained by its ability to decrease oxidative stress.

Oxidative stress is 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 [45]. In addition, lipid peroxidation may also contribute to cardiomyocyte necrosis [46]. This increased cardiomyocyte necrosis may explain the elevated levels of troponin-I in the diabetic patients included in our study, which is compatible with Rubin *et al.*, who found that patients with high HbA1c levels had elevated troponin-T levels [47].

ALA increased the mitral e'/a' ratio and LV global peak systolic strain and decreased troponin-I, which means that ALA improves left ventricular dysfunction and may decrease diabetes-induced myocardial damage in early stages of DCM. The ALA cardioprotective effect seemed to be a secondary consequence of its antioxidant properties and its ability to decrease inflammation, apoptosis, and fibrosis, as it resulted in a significant increase in glutathione level and a significant decrease in elevated levels of MDA, NO, TNF-α, Fas-L, and TGF-β gene expression.

Finally, we conclude that early detection of diabetic cardiomyopathy is of great importance, because in the early stages of diabetic cardiomyopathy, medical interventions such as α-lipoic acid could prevent or delay progression and reduce the risk of developing heart failure in individuals with diabetes mellitus.

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