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Trimetazidine Improves the Outcome of EECP Therapy in Patients with Refractory Angina Pectoris

Saad Rasool Shaker[1], Fadhil Al-Amran[2], Ghizal Fatima[3], Hayder Al-Aubaid[4], Najah R Hadi[1]

[1]Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Iraq

[2]Department of Cardiothoracic Surgery, Faculty of Medicine, University of Kufa

[3]Department of Biotechnology, Era University, Lucknow, India

[4]La Trobe University, Australia

Corresponding author: Prof. Najah R. Hadi. Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Iraq. E-mail: drnajahhadi@yahoo.com. ORCID ID:http//www.orcid.org/0000-0001-9084-591X.

ABSTRACT

Introduction: Cardiovascular disease (CAD) associated with death and disability remains a serious medical problem. In some patients the initial clinical coronary artery disease presentation is stable angina pectoris. **Aim:** The aim of the study was to evaluate the effect of EECP therapy with or without trimetazidine (TMZ) in patients with refractory angina via modulating peripheral monocyte expression of Toll like receptor2 (TLR2) and its downstream signaling. **Methods:** This is a double-blind randomized prospective study in which 88 stable refractory angina patients allocated into two groups, Enhanced External Counter Pulsation (EECP) group: included 44 patients with stable refractory angina, and were treated with EECP-Therapy. TMZ-EECP group: included 44 patients with stable refractory angina, we gave TMZ 35 mg twice daily in addition to EECP-Therapy. **Results:** TLR2 expression in peripheral monocyte investigated by flow cytometry and 8-iso-prostaglandin F2β (8-iso-PGF2 β), interleukin1β (IL-1β), heat shock protein 60 (HSP60) and monocytes chemoattractant protein-1(MCP-1) were also measured before the EECP-therapy and before giving TMZ to patients, and after 35 hours of EECP treatment (7 consecutive weeks). Inhibition in TLR2 expression in peripheral monocyte was observed among the EECP group ($P<0.05$). Inflammatory cytokine MCP-1 was remarkably decreased in both study groups but (heat shock protein 60 (HSP60), MCP-1 and interleukin-1β (IL-1β)) significantly decreased levels were observed among the TMZ-EECP group ($P<0.05$). Also, the oxidative stress biomarker 8-iso-prostaglandin F2β (8-iso-PGF2β) was decreased in both study groups but significantly decreased levels were observed among the TMZ-EECP group ($P<0.05$). TMZ and EECP therapy in patients with stable refractory angina remarkably decreased the inflammatory markers HSP60, MCP-1 and IL-1β in serum levels also the decreased levels were found in serum levels of oxidative stress marker 8-iso-PGF2β serum level. **Conclusion:** EECP-therapy decreased the expression of TLR2 on peripheral monocytes in patients with chronic stable refractory angina which yield improvement in the quality of patients' life by decreasing the frequency of angina episodes, decreasing the Short-acting nitrate use and change the exercise tolerance and distance.

Keywords: Timetizidine, Enhanced External Counter Pulsation.

1. INTRODUCTION

Cardiovascular disease (CVD) associated with death and disability remains a serious medical problem. In some patients the initial clinical coronary artery disease presentation is stable angina pectoris (1). Asymptomatic coronary artery disease in the United State (U.S.) about 6.4 million patients, and in each year develop about 400,000 new cases indicate for invasive procedures (cardiac bypass surgery and angioplasty) and/or optimal medical therapy, In the U.S. refractory angina pectoris (RAP) estimated about 300,000 to 900,000 patients. In each year about 25,000 to 75,000 of RAP new cases are diagnosed (2). Atherosclerosis is one of the inflammatory diseases. More than 150 years ago atherosclerosis inflammatory hall-marks were first described (3)and over the past 30-40 years this subject has grown widely (4). In the early 1990s, F2-isoprostanes were first discovered, in vivo, 8-Isoprostane found to reflect oxidative stress and lipid peroxidation which represented as stable of arachidonic acid end product belonging to the F-isoprostane (5). There was an association reported between 8-iso-PGF2α enhanced formation and several cardiovascular risk factors, as well as atherosclerosis (6, 7). Thus, in humans with atherosclerosis, lipopro-8-isoprostane plasma and urinary levels increased (8). MCP-1 produced by many cell types, including endothelial, fibroblasts, epithelial, smooth muscle, mesangial, astrocytic, monocytic, and

microglial cells. These cells are important for immune responses in the peripheral circulation and tissues (9). There was a link between MCP-1 and cardiovascular disease which is reported by several studies. Using MCP-1 or CCR2-deficient mice to examine atherosclerosis, it was demonstrated that the arterial lipid deposition reduced in the absence of MCP-1 or its receptor CCR2 (10 11). Heat shock proteins or stress proteins produced in many cells due to stress stimuli, such as heat shock, oxidized LDL (oxLDL), mechanical stress, infections, surgical stress, oxidants and cytokine stimulation (12).

The HSPs induce inflammatory responses in cardiovascular tissues, which are highly expressed, in the development of atherosclerosis; the HSPs may be expressed as auto-antigens (13). Levels of HSPs 60 were found to be elevated in patients with early CVD (14). The innate immune system can activate (via toll-like receptor 4) by HSP60 and also the adaptive immune system is activated (15). IL1β represented as an "inflammation gatekeeper"(16). When IL-1 type I receptor activates, it mediates a state of proinflammatory characterized by elevation of inducible nitric oxide synthase production, Endothelin 1, and other proinflammatory Chemokines, cytokines, and adhesion molecules. All of which leads to macrophage activation, endothelial and smooth muscle cell proliferation, leading to atherosclerosis progression. The role of IL-1β in atherosclerosis has long been established (17). In atherosclerotic mice model, activation of TLR2 enhanced the atherosclerotic plaque formation, which had a role in atherosclerotic occlusive disease initiation and progression (18). The TLR-2 blockade may be beneficial in cardiovascular disorders (19). TLR2 has only one pathway, MyD88-dependent pathway, and about TLR2 is more complex in activation, which makes heterodimers with TLR1 and TLR6 (20).

2. PATIENTS AND METHODS

This study is a prospective double-blind randomized control trial, Eighty-eight patients with chronic stable refractory angina were recruited from the private clinic of Dr. Prof. Fadhil Ghali Yousif, Al-Najaf Center of Cardiac Surgery, during a clinical screening procedure performed by a cardiologist, that was mandatory for all patients referred for EECP. The patients were divided randomly into two groups, after the exclusion, the patients who suffered from uncontrolled atrial fibrillation, decompensate heart failure, severe aortic insufficiency, severe peripheral arterial disease, severe hypertension, aortic aneurysm, venous disease, severe chronic obstructive pulmonary disease, epilepsy patients and those patients who were already on Monoamine oxidase inhibitors (MAOIs) treatments and having allergy with TMZ were excluded. EECP group included 44 patients with stable refractory angina and were treated with ECP-Therapy. TMZ-EECP group include 44 patients with stable refractory angina in this group patients were given Trimetazidine 35 mg twice daily in addition to EECP-Therapy.

All randomized patients that were enrolled in the study had signed informed consent and the approval for the study was granted by the Kufa University\faculty of medical ethics committees. Two blood samples were collected from each patient. The first sample was taken immediately before giving TMZ to patients and before the EECP-therapy, and the second blood sample was taken after 35 1-hour sessions of EECP therapy and then we drew 5ml of blood from a peripheral vein in each case and then divided into 2 ml of aspirated blood, blood was then put in sterile Ethylene diamine tetra acetic acid (EDTA) bottles for flow cytometry analysis of TLR2 and rest 3 ml of blood was centrifuged at 3000 ×g for 5 min to obtain serum which was then kept at -80 C to be used for the assay of 8-iso-PGF2a, IL1β, HSP60, and MCP-1.

Patient's satisfaction

Patient satisfaction was one of subjective evaluation based on the patients response to a questionnaire administered before TMZ and EECP therapy and after 35 hours of EECP treatment (7 consecutive weeks) and after treatment with TMZ. Patient satisfaction is an indicator for measuring the improvement in the quality of life, anginal pain, Short-acting nitrate use, exertional dyspnea, five-minute work and Exercise tolerance. Patient satisfaction determines whether the patients saw an improvement, worsening, or no change.

Flow cytometic analysis

Bricyte E6 flow cytometic used for measuring peripheral monocyte cell expression for TLR2. florescent Phycoerythrin PE (anti-TLR2)antibody used for 45 minutes at 4 C° blood to stain sample in a dark environment. After that the red blood cell (RBC) lysis buffer incubated with the mixture, after that the mixture washed by using phosphate buffer, then using irrelevant Isotope-matched control IgG as a control. The washed cells cell-associated fluorescence measured by Bricyte E6 flow cytometic (Mandray, China) and the data were analyzed by MR flow software.

ELISA technique

Sandwich enzyme immune assay technique was used for measuring serum level concentrations of 8-iso-PGF2a, IL1B, HSP60, and MCP-1 using a kit of Elabscience Elisa. At room temperature, 100µl serum was added to each well and incubated for 1.5 hours. After that, 100 µl of prepared biotinylated detection antibody was added to each well and then incubated at room temperature for 1 hour, and then it was aspirated and washed three times. 100 µl of HRP conjugated solution was added and then incubated for 30 minutes at room temperature again it was aspirated and washed five times. Substrate reagent as 90 µl was added and incubated for fifteen-minute at 37 degrees. Finally, 50 µl of stop solution was added. Color intensity was then measured at 450nm.

Statistical analysis

Statistical analyses were performed by using statistical package for social science (SPSS) version 20. Categorical variables were presented as number and percentage using the Chi-square test to express the association between categorical variables. Continuous variables were expressed as Mean ± stander error of the mean. Paired

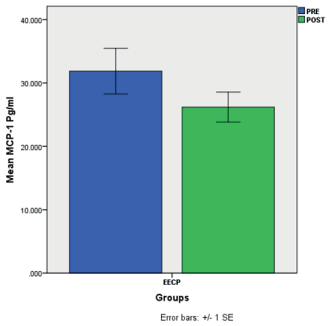

Figure 1. Effect of EECP-therapy on MCP-1 serum level, comparison between Pre-EECP therapy and Post-EECP therapy

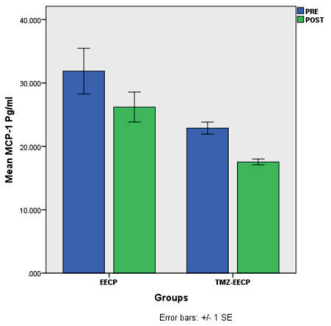

Figure 2. Effect of TMZ and EECP therapy on MCP-1 serum level, comparison between Post TMZ-EECP therapy and Post-EECP therapy

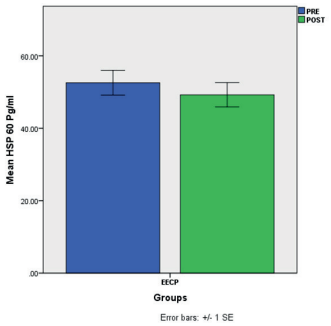

Figure 3. Effect of EECP-therapy on HSP60serum level, comparison between Pre-EECP therapy and Post-EECP therapy

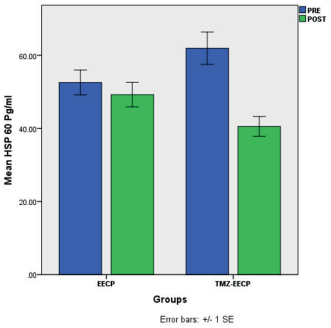

Figure 4. Effect of TMZ and EECP therapy on HSP60serum level, comparison between Post TMZ-EECP therapy and Post-EECP therapy

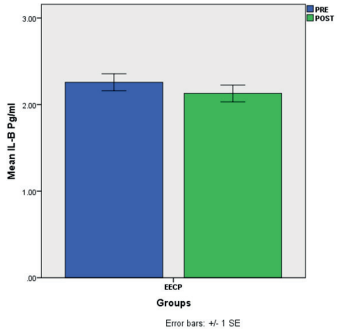

Figure 5. Effect of EECP-therapy on IL-1β serum level, comparison between Pre-EECP therapy and Post-EECP therapy

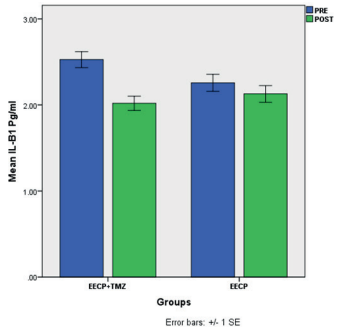

Figure 6. Effect of TMZ and EECP therapy on IL-1β serum level, comparison between PostTMZ-EECP therapy and Post-EECP therapy

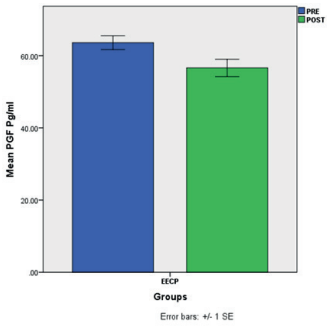

Figure 7. Effect of EECP-therapy on 8-iso-PGF2α serum level, comparison between Pre-EECP therapy and Post-EECP therapy

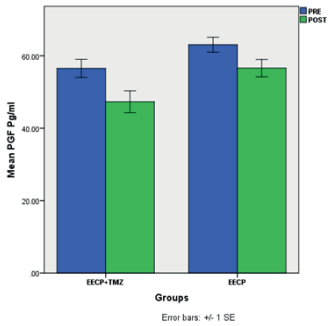

Figure 8. Effect of TMZ and EECP therapy on 8-iso-PGF2α serum level, comparison between Post TMZ-EECP therapy and Post-EECP therapy

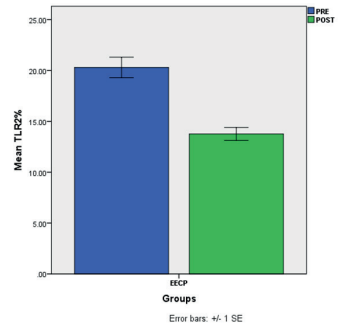

Figure 9. Effect of EECP-therapy on peripheral blood monocyte expression of TLR2, comparison between Pre-EECP therapy and Post-EECP therapy

t-test was used for comparison of means at various time point in the same group. The independent sample t-test used for comparison between two groups. P-value <0.05 was regarded as statistically significant.

3. RESULTS

All the baseline parameter of the EECP-group and TMZ-EECP group are not significant statistically regarding gender, age, smoking, history of diabetes mellitus, hypertension, drugs intake, total cholesterol, renal function test this details in Table 1

Patient's satisfaction

In this randomized study, we found in EECP-group there was 70.5% of the patients satisfy to the quality of life, anginal pain, short-acting nitrite use,excertional dyspnea, five-minute work and exercise tolerance after 35 hours of EECP treatment (7 consecutive weeks) and in TMZ-EECP group there was 88.6% of patients satisfied after TMZ 35mg twice a day with 35 1-hour sessions of EECP therapy. There is a significant difference between EECP-group and TMZ-EECP group (p-value = 0.034) in the patients satisfaction.

Effect of EECP and TMZ therapy on MCP-1 serum level

In the EECP-group of our study there was a significant decrease (P<0.05) in the serum level of MCP-1 after 35-1 hour sessions of EECP therapy (Post EECP therapy) in comparison with Pre-EECP therapy and these results shown in Figure1.

Also, we found in the TMZ-EECP group, there was a highly significant decrease (P<0.05) in the MCP-1 serum level in the post TMZ-EECP combination therapy in comparison with EECP-group (post-EECP therapy) and these results shown in Figure2.

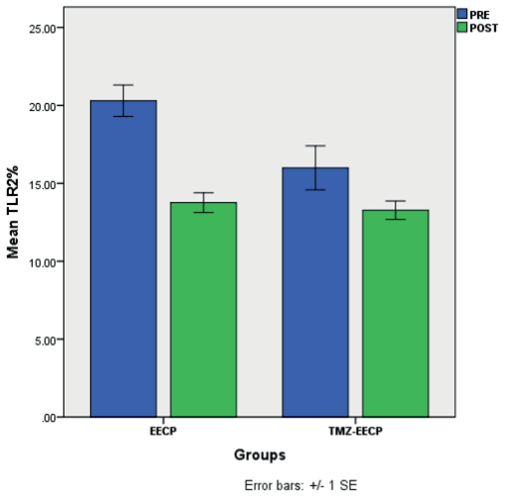

Figure 10.Effect of TMZ and EECP therapy on peripheral blood monocyte expression of TLR2, comparison between Post TMZ-EECP therapy and Post-EECP therapy

patients characteristics	TMZ-EECP	EECP	p
Male	40 (90.9%)	39 (88.6%)	N.S
Female	4 (9.1%)	5 (11.4%)	N.S
Age(years)	57.27±6.4	57.84±7.12	N.S
BMI Kg/m2	26.35±4.43	27.21±3.40	N.S
Smoker	1 (2.3%)	2 (4.6%)	N.S
X-smoking	12 (27.3%)	12 (27.3%)	N.S
Insulin	2 (4.6%)	3 (6.9%)	N.S
Oral hypoglycemic drugs	20 (45.5%)	14 (31.8%)	N.S
Aspirin	10 (22.7%)	9 (20.5%)	N.S
Clopidogril	8 (18.2%)	14 (31.8%)	N.S
B-blocker	3(6.9%)	6 (13.6%)	N.S
Angiotensin-converting enzyme inhibitors ACEI	7 (15.9%)	8 (18.2%)	N.S
Calcium channel blocker	4 (9.1%)	7 (15.9%)	N.S
Nitrate	10 (22.7%)	12 (27.3%)	N.S
Antihyperlipidemic drugs	6 (13.6%)	7 (15.9%)	N.S
Diuretics	5 (11.3%)	12 (27.3%)	N.S
Hypertension	24 (44.4%)	30 (55.6%)	N.S
Diabetes	36 (50%)	36 (50%)	N.S
B. urea mg/dl	44.75±13.961	45.82±7.146	N.S
B. sugar mg/dl	183.0357±17.708	175.2581±30.7	N.S
S. creatinine	0.968±0.0174	0.9621±0.111	N.S
Cholesterol	185.9286±40.295	166.9167±34.486	N.S
TG	167.55±25.129	180.5556±74.061	N.S
EF	51.42±6.619	51.9±9.939	N.S

Table1 Demographic characteristics of participated patients

Effect of EECP and TMZ therapy on HSP60 serum level

In EECP-group of the present study, there was a non-significant decrease (P<0.05) in the HSP60 serum level after 35-1 hour sessions of EECP therapy (Post EECP therapy) in comparison with Pre-EECP therapy and these results shown in Figure 3, while in the TMZ-EECP group, there was a significant decrease (P<0.05) in the HSP60 serum level in post TMZ-EECP combination therapy in comparison with EECP-group (post-EECP therapy) and these results shown in Figure 4.

Effect of EECP and TMZ therapy on IL-1β serum level

In EECP-group of this randomized study, there was a non-significant decrease (P<0.05) in the IL-1βserum level after 35-1 hour sessions of EECP therapy (Post EECP therapy) in comparison with Pre-EECP therapy and these results shown in Figure 5, while in the TMZ-EECP group, there was a significant decrease (P<0.05) in the IL-1β serum level in post TMZ-EECP combination therapy in comparison with EECP-group (post-EECP therapy) and these results shown in Figure 6.

Effect of EECP and TMZ therapy on 8-iso-PGF2α serum level

In EECP-group, there was a significant decrease (P-value <0.05) in the serum level of 8-iso-PGF2α after 35-1 hour sessions of EECP therapy (Post EECP therapy) in comparison with Pre-EECP therapy and these results shown in Figure 7. In the TMZ-EECP group, there was a significant decrease (P-value <0.05) in the serum level of 8-iso-PGF2α in post TMZ-EECP combination therapy in comparison with EECP-group (post-EECP therapy) and these results shown in Figure 8.

Effect of EECP and TMZ therapy on peripheral blood monocyte expression of TLR2

In EECP-group of the current study, there was a significant decrease (P<0.05) in the peripheral blood monocyte expression of TLR2 after 35-1 hour sessions of EECP therapy (Post EECP therapy) in comparison with Pre-EECP therapy and these results shown in Figure 9, but in TMZ-EECP group, there was a non-significant decrease (P<0.05) in the peripheral blood monocyte expression of TLR2 in post TMZ-EECP combination therapy in comparison with EECP-group (post-EECP therapy) and these results shown in Figure 10.

4. DISCUSSION

Enhanced external counter pulsation is a non-invasive therapy, for CAD and for patients with RAP who fail to respond to standard revascularization procedures and aggressive pharmacotherapy. Data from the International Patient Registry (IEPR) demonstrate that angina episodes and nitrate usage are decreased by the effect of EECP, and exercise tolerance increases in patients with RAP (21,22). The anti-ischemic benefits occur early and are sustained up to 5 years in patients with a favorable initial response (23). In the EECP-group of the present study we found that there was a significant decrease (P<0.05) in the serum level of MCP-1 in the post-EECP therapy in comparison with a pre-EECP therapy, these results agreed with Braith et al. 2010, study which reported that, the plasma levels of TNF-α, hsCRP and MCP-1decreased after 35 sessions of EECP treatment (24, 25). Our study findings are in concordance with Casey et al. 2008 who reported that the MCP-1plasma levels are reduced after EECP therapy (26). In the coronary artery of atherosclerotic patients, the serum level of MCP-1 seen to be high (27, 28). Oxidized-LDL enhanced the expression of MCP-1 from macrophages, vascular smooth muscle cell, and endothelial cells; the MCP-1 expression is time and level-dependent manner (29). The EECP mechanism as anti-inflammatory action mostly related to the intermittent bouts of shear stress created with each inflation/deflation cycle of the cuffs. Shear stress enhanced endothelial-derived nitric oxide (NO) synthesis and release (30). Besides the NO vasodilatation effect, it also had an anti-inflammatory and anti-atherosclerotic role via reducing the VCAM-1 expression and inhibiting the MCP-1 expression (31). In this randomized study, we also found there was a highly significant decrease (P<0.05) in the serum level of MCP-1

in the post TMZ-EECP group in comparison with a post EECP-group, Trimetazidine cardio-protective effect like oxidative stress reduction, which decreased lipid oxidation and inhibited monocyte/macrophage stimulation for chemokine and inflammatory cytokines production (32). Also, TMZ reduced the NO inactivation rate by stimulating the endothelial function, The anti-inflammatory and anti-atherosclerotic role of NO by its inhibitory effect on the MCP-1 expression and by reducing the expression of VCAM-1 (33). Finally, TMZ decreases the vascular cell adhesion molecules-1 and MCP-1 by its inhibitory effect on NF-kB (34). In the EECP-group of the present study we found that there was a non-significant decrease ($P<0.05$) in the serum level of IL-1β in the post-EECP therapy in comparison with a pre-EECP therapy but there was a significant decrease in the serum level of IL-1β in post TMZ-EECP group in comparison with post EECP-group and this agreed with Kuralay et al. which supposed that the trimetazidine inhibits the inflammatory markers like nitric oxide products (nitrite and nitrates), IL-1β, IL-6 and TNF alpha (35). IL1β represented as an "inflammation gatekeeper" (15). Which activates macrophage, endothelial and smooth muscle cell proliferation, leading to atherosclerosis progression (17). Zhang et al. approved that the trimetazidine increased the level of Nrf2/HO-1, Nrf2 pathway plays a major role in inflammation, Nrf2 is negatively regular at NF-kB, NF-kB is a key mediator for inflammation which induce Chemokines and other inflammatory cytokines like (IL-1B, IL6, TNF-a) (34). In the current there was a non-significant decrease ($P<0.05$) in the serum level of HSP-60in the post-EECP therapy in comparison with a pre-EECP therapy and there was a significant reduction in serum level of HSP-60 in post TMZ-EECP group in comparison with the post-EECP treated group. HSP60 level elevated in patients with early CVD (36). The innate immune system can activate (via toll-like receptor4) by HSP60and also the adaptive immune system activated (37). The synergism effect of EECP and TMZ on the stress markers such as oxidative stress, endothelial dysfunction and inflammatory markers (i.e. stress markers enhance HSP60 release) (38, 39) cause a significant decrease in HSP60 serum level in TMZ-EECP group when compare with EECP-group in patients with stable refractory angina of our study. In this study 8-iso-PGF2 serum level significantly decrease ($P<0.05$) in post-EECP therapy in comparison with a pre-EECP therapy, these results were in agreement with Braith et al. 2010, which observed that after 35 sessions of EECP treatment, the 8-iso-PGF2a serum level decreased (25). Also there was a highly significant reduction in the serum level of 8-iso-PGF2a in post TMZ-EECP group in comparison with the post-EECP group. In cardiovascular diseases, the 8-iso-PGF2 represented as the most valid systemic oxidative stress biomarker (40). Trimetazidine can bind to mitochondria and it significantly increases the rate of glucose oxidation and reduces the rate of fatty acid oxidation (41). In this study the peripheral blood monocyte expression for TLR2 significantly decreased ($P<0.05$) in the post-EECP therapy in comparison with a pre-EECP therapy while there was a non-significant reduction in peripheral blood monocyte expression of TLR2 in post TMZ-EECP group in comparison with the post-EECP group. TLR2 blockade may be beneficial in cardiovascular disorders (19). TLR2 blockade reduces the infarct size and maintains heart function (42). The endogenous ligands of TLR2 are oxLDL (43), Oxidized phospholipids (44), HSPs (45). Braith et al. improved the effect of EECP on lipid peroxidation (TLR2 endogenous ligand) (25). TLR2 has only one pathway, MyD88-dependent pathway, and about TLR2 is more complex in activation, which made heterodimers with TLR1 and TLR6 (20).

- **Author's contribution**: Each author gave substantial contribution in acquisition, analysis and data interpretation. Each author had a part in preparing article for drafting and revising it critically for important intellectual content. Each author gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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