Journal of Kerbala University , Vol. 14 No.2 Scientific . 2016

Effect of carvedilol on echocardiographic fraction shortening, troponin I, malondialdehyde in trastuzumab treated females with breast cancer

تأثير الكارفيديلول على صدى القلب لقياس الجزء المقذوف ، بروتين التروبونين نوع (I)، عامل التأكسد المالون ثنائي الالديهايد للإناث اللواتي يستخدمن الترستوزوماب المصابات بسرطان الثدي

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Abstract

Background: Trastuzumab therapy is effective in HER2 positive breast cancer patients, but it is highly complicated by cardiotoxicity.

Objective: To elucidate the possible effects of carvedilol on echocardiographic fraction shortening, serum troponin I and serum malondialdehyde in trastuzumab treated females with HER2 positive breast cancer.

Patients and Methods: A total of twenty six females with HER2 positive breast cancer were enrolled in this study. The patients were randomized into two groups, thirteen patients in each group. Group I included patients who received trastuzumab therapy for 8 cycles with 21 days apart. Group II included patients who treated with trastuzumab therapy with carvedilol 3.125 mg, orally, twice daily dose for 8 cycles. Echocardiography was used to measure fraction shortening at zero time, 4^{th} and 8^{th} cycles in both groups. Serum troponin I and malondialdehyde were measured at zero time, 2^{nd}, 4^{th}, 6^{th} and 8^{th} cycles in both groups.

Results: Treatment with trastuzumab therapy caused a significant decrement in echocardiographic fraction shortening at an only 8^{th} cycle in comparison to baseline readings ($P < 0.05$).Combined trastuzumab plus carvedilol caused a significant increase in echocardiographic fraction shortening compared with that of trastuzumab therapy group ($P < 0.05$). Regarding serum troponin I and malondialdehyde, trastuzumab therapy caused a significant increment in both these markers in comparison to baseline readings ($P < 0.05$). Combined trastuzumab plus carvedilol caused significant decrement in troponin I and malondialdehyde compared with that of trastuzumab therapy group ($P < 0.05$).

Conclusion: Carvedilol causes significant increment in echocardiographic fraction shortening and significant decrement in serum troponin I and malondialdehyde in trastuzumab treated patients.

Key words: Carvedilol, Trastuzumab, Cardiotoxicity, Fraction shortening, Troponin I, MDA

الخلاصة :

علاج التراستوزوماب يعتبر فعالا للمريضات المصابات بسرطان الثدي نوع هير2 الايجابي ولكنه مصحوبا بالتسمم في عضلة القلب

الهدف: لتوضيح التأثيرات الممكنة لعقارالكارفيديلول على فحص صدى القلب لقياس الاختصار الجزئي, بروتين التروبونين نوع (I) , وعامل التأكسد المالون ثنائي الالديهايد للمريضات المصابات بسرطان الثدي نوع هير2 الايجابي.

المرضى وطريقة العمل: 26 مريضة مصابة بسرطان الثدي شاركت في الدراسة الحالية , وزعت المريضات بصورة عشوائية إلى مجموعتين , 13 مريضة في كل مجموعة.

المجموعة الأولى: شملت المريضات اللواتي عولجن بالنظام العلاجي المعتمد على التراستوزوماب لثمان دورات علاجية مع فاصل زمني قدره 21 يوما بين كل دورة ودورة.

المجموعة الثانية: شملت المريضات اللواتي عولجن بنفس النظام العلاجي + 3,125 ملغم كارفيديلول مرتان يوميا , عن طريق الفم لمدة ثمان دورات. أجرت كل مريضة فحص صدى القلب لقياس الاختصار الجزئي في وقت الصفر من الدراسة وبعد الدورة الرابعة والثامنة. تم سحب عينات الدم في وقت الصفر وبعد الدورة الثانية والرابعة و السادسة والثامنة وتم قياس بروتين التروبونين نوع (I) , وعامل التأكسد المالون ثنائي الالديهايد.

النتائج: سبب العلاج مع تراستوزوماب نقصان معنوي في الاختصار الجزئي في فحص صدى القلب في الدورة العلاجيه الثامنه بالمقارنة مع القراءات الاوليه ($P< 0.05$) وسبب زيادة معنويه في مستوى المصل لكل من بروتين التروبونين نوع (I) , و عامل التأكسد المالون ثنائي الالديهايد بالمقارنة مع القراءات الاولية ($P<0.05$).

فيما يتعلق بالمجموعة الثانية (تراستوزوماب مع الكارفيديلول)، كانت هناك زيادة معنوية في الاختصار الجزئي في فحص صدى القلب وإنخفاض معنوي في مستوى المصل لكل من بروتين التروبونين نوع (I) و عامل التأكسد المالون ثنائي الالديهايد مقارنة بنفس الفحوصات في مجموعة العلاج مع التراستوزوماب ($P<0.05$).

الاستنتاجات:

الكارفيديلول سبب زيادة معنوية في الاختصار الجزئي في فحص صدى القلب وإنخفاض معنوي في مستوى المصل لكل من بروتين التروبونين نوع (I) و عامل التأكسد المالون ثنائي الالديهايد لدى المريضات اللواتي عولجن بالتراستوزوماب

Introduction

Breast cancer is a malignant tumor that begins in the breast cells. It is the most commonly diagnosed cancer and it represents the second leading cause of cancer death among females[1]. Cells of the breast have many different types of receptors, one of the most important receptor being: human epidermal growth factor receptor (HER2). 25-30% of breast cancers are overexpressing the HER2 protein[2]. Trastuzumab is a humanized monoclonal antibody directed against the HER2 receptor[3]. Since 2006 concurrent treatment with trastuzumab is truly the standard of care for patients with early HER2 positive breast cancer[4]. Trastuzumab binds to HER2 proteins with high affinity and blocks the effects of neuregulin-1 (NRG-1). Generally, NRG-1 binds to and activates the HER4 protein, which is then primed for binding to HER2 protein. Binding of NRG-1 to HER2 protein initiates cell survival pathways that maintain cardiac function and inhibit apoptosis. This binding initiates an alteration in mitochondrial respiration, leading to decrease the production of reactive oxygen species (ROS) and increase cell survival. Additionally, NRG-1 signalling is able to reveal cardioprotective properties through the activation of FAKs (focal adhesion kinases). FAK is essential in maintaining the function and structure of sarcomeres[5].Furthermore, the increased stress on the cardiomyocyte results in the upregulation of circulating angiotensin II. This upregulation has two detrimental effects on the cardiomyocyte. First, angiotensin II is a potent inhibitor of NRG. The second detrimental effect is that angiotensin II leads to the activation of NADPH oxidase[6], which responsible for production of ROS. Trastuzumab-induces cardiotoxicity (TIC) is reported to be reversible and is not dose-related. Nevertheless, some cases have resulted in thrombosis, disabling HF, and/or death[7]. Early detection of patients at risk for cardiotoxicity represents a main goal for oncologists and cardiologists, by allowing for the definition of personalized interventions or antineoplastic therapeutic strategies. Most of the approaches frequently used in clinical practice such as echocardiography denoted low predictive power and low diagnostic sensitivity in detecting subclinical damage of cardiomyocyte. The use of some other techniques, like endomyocardial biopsy, is uncooperative in clinical practice due to the invasiveness of the techniques. Therefore, there is growing expectation for newer, cost effective and noninvasive diagnostic tools for the early recognition of patients liable to developing drug induced cardiotoxicity[8]. Uses of easily measurable biomarkers in blood, like brain natriuretic peptides (BNP) and C-reactive protein (C-RP), have been assessed in animal models and in clinical studies. Many studies have linked the severity of myocardial inflammation or cardiac ventricular wall stress caused by remodeling with higher levels of these circulating markers[9,10]. Carvedilol is a nonselective β-blocker drug with additional vasodilating, anti-inflammatory and antioxidative properties. Carvedilol is used in the management of hypertension and angina pectoris, and was the first drug among β-blockers to be approved in the management of CHF in adults[11]**.** Accumulating evidence has revealed that carvedilol protects against chemotherapy induced cardiotoxicity through its antioxidant properties[12]. The goal of this

study is to assess the value of the use of carvedilol in the prevention of TIC in female patients receiving trastuzumab for breast cancer that over expressed HER2 receptor.

Patients and methods

Patients: The study sample involved females who attended the oncology unit in Al-Sadar medical city in Al-Najaf Al-Ashraf Governorate from 1st of April 2013 to the 25thof July 2014 with established new diagnosis of HER2 positive breast cancer. Exclusion criteria were patients with the past-medical history of heart disease, renal failure, diabetes mellitus or thyroid diseases. Twenty six patients were enrolled in this study and divided randomly into 2 groups, 13 patients per group. In group I patients were treated with trastuzumab regimen for 8 cycles with 21 days interval. In group II patients were treated with trastuzumab regimen plus carvedilol 3.125 mg administered orally twice daily for 8 cycles with 21 days interval. Each patient was informed about treatment. The ethical committees of Al-Nahrain College of medicine approved the study protocol. Carvedilol was manufactured by TAD Pharma GmbH, Germany. Batch NO. N73094.

Echocardiography (ECHO): Each individual included in the study (both patients groups), underwent echocardiography, at zero time, 4th and 8th cycles. The study was performed for determining left ventricular fraction shortening (LVFS). LVFS reflects the relative change of left ventricular internal dimension throughout the cardiac cycle; it is measured as the difference ratio between end diastolic and end systolic internal diameters to the end diastolic internal diameter. To obtain the percent of fraction shortening multiply this by 100. It is the most commonly applied M-Mode derived a measure of left ventricular systolic function [13].

Collection of Blood Samples: Five ml of blood was collected at zero time and at 2nd, 4th, 6th and 8th cycles for evaluation of TIC based on changes of the serum troponin I (CTnI) and MDA biomarkers. Each blood sample was centrifuged at 2500 rpm for 15 minutes, and then serum was collected and frozen at - 80 until measurement.

Measurement of Troponin I: Using commercially available human cardiac (CTnI) ELISA kit (catalog number CSB-E05139h) from Cusabio Biotech Co., LTD.

Measurement of Malondialdehyde (MDA):
The level of serum MDA was determined by a modified procedure described by Buege and Aust in 1978[14].

Statistical Analysis:
Statistical analyses were performed using SPSS 16.0 for windows.lnc. Data of quantitative variables were expressed as mean ± SEM. Differences in each variable through treatment cycles in the same group were compared using paired-sample Student's t-test. Unpaired-sample Student's t-test was used for the comparisons between the two groups variable. In all tests, $P<0.05$ was considered to be statistically significant.

Results:

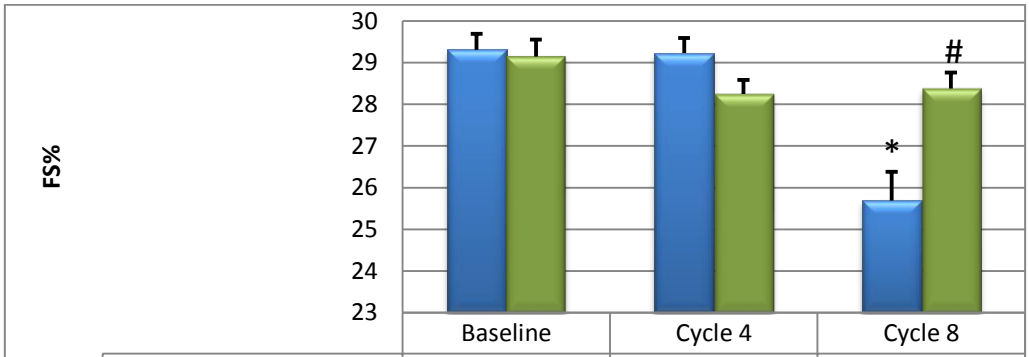
Anthropometry: There was no significant difference in anthropometric data of the two patients groups included in this study as shown in table (1).

Table (1): Anthropometric data for all included patients in this study.

Anthropometric data	Mean± SEM (Group I)	Mean± SEM (Group II)
Age (year)	38.38± 2.27	41.07± 2.08
Weight (kg)	71.61±1.87	67.61± 1.48
Height (cm)	158.84±2.35	161.92± 1.35
Body Surface Area (m^2)	1.73±0.03	1.71± 0.019
Body Mass Index (kg/m^2)	28.02± 0.85	25.87± 0 .67

Effect of Different Treatment Regimen on Echocardiographic Fraction Shortening (FS %):

In comparison with baseline levels, there was a significant reduction in FS % at only the 8 cycle in trastuzumab regimen ($p< 0.05$) as shown in figure (1). In comparison between treatment groups, there was no significant difference ($P > 0.05$) in FS % at baseline and after 4 cycles of treatment as shown in figure (1). At 8 cycles of treatment FS % of group II was significantly ($P < 0.05$) higher than that of group I as shown in figure (1).



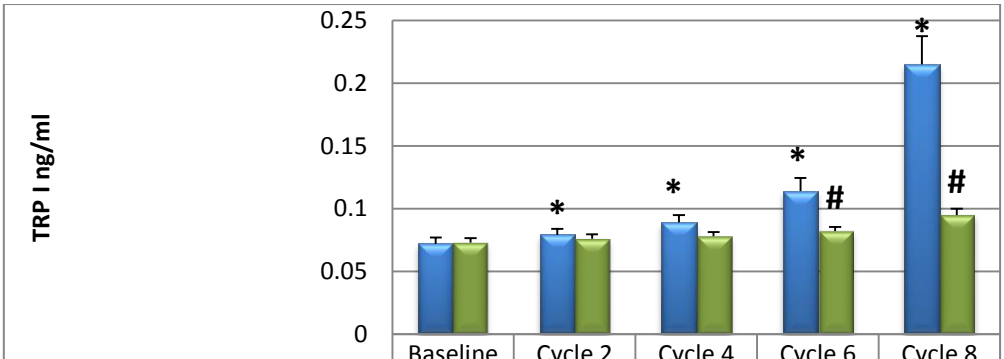
	Baseline	Cycle 4	Cycle 8
Trastuzumab	29.31	29.23	25.69
Trastuzumab+Carvedilol	29.15	28.25	28.38

* P<0.05 compare to baseline values of the same treatment group.
# P<0.05 compare to group I

Figure (1): Mean± SEM values of echocardiographic fraction shortening (%) at baseline and after 4 and 8 cycles in both groups (trastuzumab based regimen, n=13 and trastuzumab plus carvedilol, n=13).

Effect of Different Treatment Regimens on Serum Troponin I Level:

In comparison with baseline levels, there was significant increment in serum CTnI level (ng\ml) after 2, 4, 6 and 8 cycles in trastuzumab regimen group ($p< 0.05$) as shown in figure (2).In comparison between treatment groups, there was no significant difference ($P > 0.05$) in serum CTnI level at base line and after2 and 4 cycles of treatment as shown in figure (2).After 6 and 8 cycles of treatment, serum CTnI level of group II was significantly ($P < 0.05$) lower than that of group I as shown in figure (2).



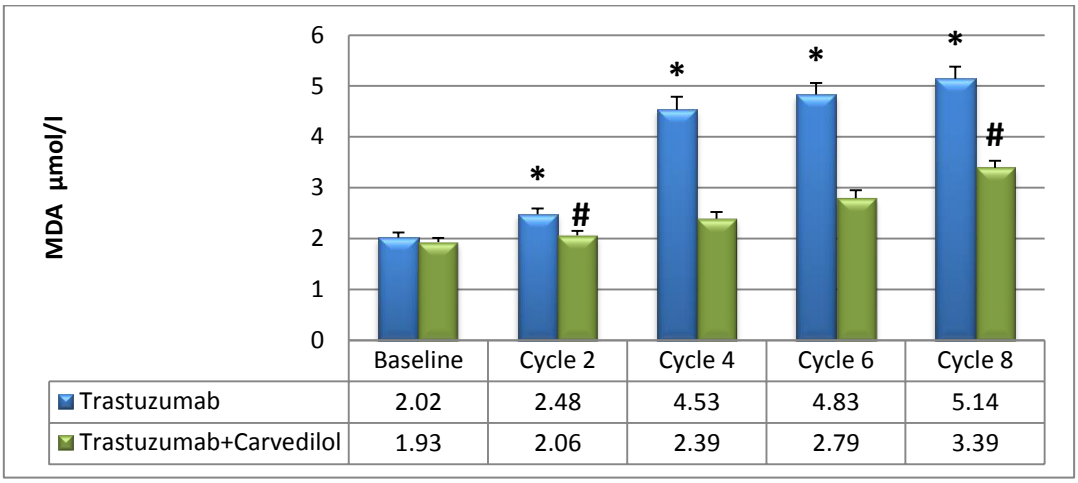
	Baseline	Cycle 2	Cycle 4	Cycle 6	Cycle 8
Trastuzumab	0.072	0.079	0.089	0.114	0.215
Trastuzumab+Carvedilol	0.073	0.076	0.078	0.082	0.095

* P <0.05 compare to baseline values of the same treatment group.
# P <0.05 compare to group I

Figure (2): Mean± SEM values of serum CTnI at baseline and after 2, 4, 6 and 8 cycles in both groups (trastuzumab based regimen, n=13 and trastuzumab plus carvedilol, n=13).

Effect of Different Treatment Regimens on Serum MDA Level:

In comparison with baseline level, there was significant increment in serum MDA level (mg\l) after 2, 4, 6 and 8 cycles in trastuzumab regimen group ($p< 0.05$) as shown in figure (3).In comparison between treatment groups, there was no significant difference ($P > 0.05$) in serum MDA level at base line of treatment as shown in figure (3). After 2,4,6 and8 cycle of treatment, serum MDA level of group II was significantly ($P < 0.05$) lower than that of group I as shown in figure (3).



* P <0.05 compare to baseline values of the same treatment group.
# P <0.05 compare to group I

Figure (3): Mean± SEM values of serum malondialdehyde (MDA) at baseline and after 2, 4, 6 and 8 cycles in both groups (trastuzumab based regimen, n=13 and trastuzumab plus carvedilol, n=13).

Discussion:

The use of trastuzumab in HER2 positive breast cancer has significantly improved response rates and enhanced survival in female with early-stage and metastatic disease[3]. However, the high incidence of cardiotoxicity, up to a third of females treated with trastuzumab might develop a cardiotoxicity[15,16], has produced great concern regarding its use. Indeed, the occurrence of cardiotoxicity restricts the selection of possible oncological therapies to those considered less aggressive and as a result less effective[17]. Patients who develop heart problems when treated with trastuzumab might have to discontinue this treatment, which could affect their chances of cure.

Effect of Trastuzumab Based Regimen on Clinical and Biochemical Parameters of the Present Study:

In the present study, there was no significant change in the FS % after 4 treatment cycles in comparison to baseline values ($P> 0.05$). Trastuzumab caused significant reduction in FS % after 8 treatment cycles in comparison to baseline values ($P < 0.01$). This finding supports previous animal experiments demonstrating that fraction shortening significantly declined from a mean of 32% in control animals to a mean of 23% in trastuzumab treated animals[18]. The mechanism beyond this effect is thought to block cardiomyocyte HER2 signalling. So that trastuzumab interfering with normal growth, repair, counteraction of undue sympathetic tone and survival of cardiomyocytes[19,20].

Trastuzumab produced highly significant increase in serum CTnI level in comparison to the baseline readings ($P < 0.01$). Several studies revealed the same result after one month and three month respectively[21,22]. In our study the timing of detectable serum CTnI appeared to precede the maximal decline in LVFS at cycle 8, for example trastuzumab produced highly significant increase in serum CTnI level at cycle 2 in comparison to the baseline readings. This finding is similar to that demonstrated in previous studies, where the CTnI increased soon after chemotherapy is a strong

predictor of myocardial injury and poor cardiological outcome, with the highest risk observed in patients showing a persistent (1 month) CTnI increase[23,24,15.]

The mechanism for CTnI elevation after chemotherapy can be speculated that subclinical cardiomyocyte damage may arise. Because the mechanism underlying TIC is considered different from that of anthracyclines, elevation of this biomarker during trastuzumab therapy further complicates our knowledge about cardiotoxic effects of anticancer treatment[25,26]. However, CTnI was observed early after treatment with trastuzumab, allowing us to identify the female patients at most hazard after the first two cycles of treatment. A possible explanation for CTnI elevation during trastuzumab treatment is blocking of HER2 receptors, expressed on cardiomyocytes, resulting in the loss of survival pathways that mediated by HER2. These pathways appear to have a protective effect on cardiac function, because they generally blunt the effects of stress signaling pathways [26].

Trastuzumab caused highly significant increase in serum MDA level in comparison to baseline values ($P < 0.01$). This finding is in consistency with that of Dirican *et al*. (2014) and Keith *et al*. (1998) [27,28].

Effect of Carvedilol on Clinical and Biochemical Parameters of the Present Study:

Carvedilol produced significant increment in FS % in comparison to that of trastuzumab based regimen group ($P < 0.05$). The mechanism beyond these effect are that carvedilol is positioned to inhibit a number of pathological processes that responsible for the progression of heart failure, including: reduction of heart rate, preload and afterload; inhibition of the sympathetic nervous system and the renin-angiotensin system; scavenging oxygen radicals; suppression of pathological organ remodeling[29].

CTnI was decreased in a highly significant manner by carvedilol in comparison to that in trastuzumab based treated group ($P < 0.01$). This finding is in agreement with that revealed by El-Shitany *et al*. (2012) and Al-Rekabi *et al*. (2012) but with doxorubicin rather than trastuzumab[30,31]. Carvedilol caused highly significant reduction in serum MDA level in comparison to trastuzumab treated group ($P < 0.01$). This result is in agreement with Castro *et al*. (2005)[32]. The suggested mechanism beyond this result may be due to carvedilol prevents lipid peroxidation in the membrane of cardiomyocytes and thus protects the cells against the injuries caused by free oxygen radicals[29].

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