Effect of Valsartan on Echocardiographic Ejection Fraction, Brain Natriuretic Peptide and Malondialdehyde in Trastuzumab Treated Females with Breast Cancer

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ABSTRACT

Trastuzumab, a monoclonal antibody, is effective in HER2 positive breast cancer patients but cardiotoxicity is problem. The aim of this study was to clarify the possible effects of Valsartan on echocardiographic ejection fraction and cardiac biomarkers (serum brain natriuretic peptide and malondialdehyde) in trastuzumab treated females with breast cancer over expressing HER2 receptor. Twenty six female patients with new diagnosis of HER2 positive breast cancer were randomly assigned to 8 cycles trastuzumab treatment (group I, n= 13) or trastuzumab plus Valsartan 40 mg (group II, n= 13). Echocardiography ejection fraction was analyzed at base line, 4th and 8th cycles in both groups. Serum malondialdehyde and brain natriuretic peptide were analyzed at base line, 2nd, 4th, 6th and 8th cycles in both groups. Treatment with trastuzumab caused significant decrease in echocardiographic ejection fraction at 8th cycle treatment in comparison to baseline readings (P < 0.05). A significant increase in echocardiographic ejection fraction was obtained by group II (trastuzumab plus Valsartan group) in comparison with that of trastuzumab therapy group (P < 0.05). Regarding serum brain natriuretic peptide and Malondialdehyde, trastuzumab therapy caused significant increase in both these markers compared with the baseline readings (P < 0.05). Combined trastuzumab plus Valsartan caused significant decrease in serum brain natriuretic peptide and Malondialdehyde compared with that of trastuzumab therapy group (P < 0.05). In trastuzumab treated patients, Valsartan causes significant increase in ejection fraction with significant decrease in both serum brain natriuretic peptide and malondialdehyde.

Keywords: BNP, Cardiotoxicity, Ejection fraction, MDA, Trastuzumab, Valsartan.

INTRODUCTION

Breast cancer represents the most common type of cancer in women worldwide and it is the second leading cause of cancer death. In about 25–30% of breast cancers, the human epidermal growth factor receptor 2 (HER 2) gene is amplified and the HER2 receptor over expressed, which giving higher aggressiveness in breast cancers. Trastuzumab is a monoclonal antibody directed against the HER 2 receptor which has proven powerful in treatment of HER 2 positive breast cancers with decreasing recurrence and mortality by approximately 50% and 33%, respectively. However, it may induce cardiac toxicity, especially when given in combination with anthracyclines. Although this toxicity reported to be reversible; some cases have resulted in disabling HF, and/or death. The mechanism by which this complication takes place connected with the role of the HER2 gene in the embryonic and postnatal heart. In fact, HER2 protein when exposed to oxidative stress triggers a protective intracellular signaling pathway (neuregulin, HER2–HER4 heterodimeric receptors). Its loss, on the other hand, activates the apoptotic cascade. Furthermore, NRG-1 signaling pathway can activate focal adhesion kinases (FAK), which is important in maintaining the function and structure of sarcomeres. Moreover, the cardiac dysfunction caused by oxidative stress lead to up regulation of angiotensin II enzyme (ANG II). ANG II up regulation leads to production of ROS through activation of NADPH oxidase. Finally, the use of trastuzumab can potentiate cardiomyocyte damage through a dual-hit mechanism, which includes inhibition of the neuregulin-1 survival signaling pathway and angiotensin II-induced activation of NADPH oxidase, with the ability to further increase reactive oxygen species production. Prior to starting trastuzumab therapy, a baseline measurement of LVEF should be done for all patients in order to establish their left ventricular function. Currently, clinical examination and sequential LVEF measurements have significant limitations, namely the fact that a decrease in LVEF represents a comparatively late stage of cardiac dysfunction. Earlier diagnosis of subclinical cardiotoxicity could lead to identifying, intervening, and preventing late cardiac risk outcomes. There is a powerful research in this region, especially in what involve the role of cardiac biomarkers. Cardiac biomarkers are substances that released into the circulation when the cardiomyocyte is damaged. A multi-marker approach is documented to be useful in early detection of myocardial injury. Brain natriuretic peptide (BNP) is found in the cardiac ventricles and atria and high plasma concentrations are present in patients with ventricular hypertrophy and CHF. Malondialdehyde (MDA) is one of the most continually used indicators of lipid peroxidation. Serum MDA was
found to be elevated in patients with cardiac damages so can confirms the predictive results in multi-marker setting.\textsuperscript{12} Valsartan is a drug that selectively inhibits angiotensin receptor (AT1). This of drugs was developed following the angiotensin-converting enzyme (ACE) inhibitors, as an option to provide more specific effects on the renin angiotensin system (RAS).\textsuperscript{13} Valsartan interact with the angiotensin receptor whereby selectively inhibiting its physiological actions. The RAS adjusts blood pressure through its effect on extracellular fluid volume, sodium balance, and renal and systemic vascular resistance. The final step of the RAS pathway is the activation of the angiotensin receptors. By blocking the action of the AT1 receptor, Valsartan leads to reduction in blood pressure (BP), as well as inhibits sympathetic outflow, improves renal function, decreases vascular smooth muscle contraction, and also leads to declining in progression of atherosclerosis lesions.\textsuperscript{13,14}

Valsartan was firstly approved in Europe in 1996 for the treatment of hypertension in adults. Additionally, it has been shown to have cardio protective effect, with reduced morbidity and mortality in patients with heart failure and following myocardial infarction.\textsuperscript{15,16} In general these effects are equivalent to those achieved with ACE inhibitors, with the additional advantage of a lower incidence of undesirable effects such as cough and angioedema.\textsuperscript{15}

Patients and methods

Patients
From 1\textsuperscript{st} of April 2013 to the 25\textsuperscript{th} of July 2014, female patients with new diagnosis of HER2 positive breast cancer at the oncology unit in Al-Sadar medical city in Al-Najaf Al-ashraf Governorate were studied. Exclusion criteria were patients with thyroid disease, past history of heart disease, renal failure, and diabetes mellitus. Twenty six patients were randomly assigned to 8 cycles trastuzumab treatment (group I, no= 13) or trastuzumab plus Valsartan 40 mg (group II, no= 13). Valsartan was administered orally single daily dose for 8 cycles. Each patient was informed about treatment. The protocol for this study was approved by ethical committees of Al-Nahra in College of Medicine. It was manufactured by Novartis Pharma AG, Basle, Switzerland. Batch NO. B0504.

Echocardiography (ECHO)

In both patients groups, each individual underwent echocardiography for determining LVEF at baseline, 4\textsuperscript{th} and 8\textsuperscript{th}treatment cycles.

Collection of Blood Samples

Four ml of blood was collected at baseline, 2\textsuperscript{nd}, 4\textsuperscript{th}, 6\textsuperscript{th} and 8\textsuperscript{th}treatment cycles for assessment of cardiotoxicity induced by trastuzumab therapy based on changes of the serum MDA and BNP biomarkers. Each blood sample was centrifuged, and the obtained serum was frozen at - 80 until measurement.

Measurement of BNP

Using human BNP ELISA kit (catalog number CSB-E07970h). This kit is commercially available from Cusabio Biotech Co., LTD.

Measurement of MDA

Malondialdehyde was estimated by the method of Buege and Aust\textsuperscript{17} in which based on the reaction of MDA with thiobarbituric acid (TBA) to form a red MDA-TBA complex which can be quantitated spectrophotometrically:

$$\text{Absorbance at 532 nm}$$

$$\text{The concentration of MDA} = \frac{\text{mean absorbance}}{\text{extinction coefficient}} \times D \times V$$

Where:
- $E$: extinction coefficient 1.56 x 105 M$^{-1}$Cm$^{-1}$.
- $D$: dilution factor = ______________________
- $V$: volume of the sample

Statistical Analysis

Statistical analyses were performed using SPSS 16.0. Data of quantitative variables were presented as mean ± SEM. Differences in each variable in the same group were compared using paired-sample Student’s t-test. Unpaired-sample Student’s t-test was performed to assess the difference between the two groups. In all tests, P<0.05 was considered to be statistically significant.

RESULTS

Anthropometry

Between the two groups included in this study, there was no significant difference in anthropometric data as shown in table (1).

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

<table>
<thead>
<tr>
<th>Mean± SEM (Group I)</th>
<th>Mean± SEM (Group II)</th>
<th>Anthropometric data</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.61± 1.94</td>
<td>38.38± 2.27</td>
<td>Age (year)</td>
</tr>
<tr>
<td>70.92± 1.60</td>
<td>71.61± 1.87</td>
<td>Weight (kg)</td>
</tr>
<tr>
<td>158.23± 1.71</td>
<td>158.84± 2.35</td>
<td>Height (cm)</td>
</tr>
<tr>
<td>1.72± 0.02</td>
<td>1.73± 0.03</td>
<td>Body Surface Area (m$^2$)</td>
</tr>
<tr>
<td>28.43± 0.87</td>
<td>28.02± 0.85</td>
<td>Body Mass Index (kg / m$^2$)</td>
</tr>
</tbody>
</table>

Effect of the Two Treatment Regimen on Echocardiographic Ejection Fraction (%)

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

*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 ejection fraction (%) at baseline and after 4 and 8 cycles in both groups (trastuzumab therapy, n=13 and trastuzumab plus Valsartan, n=13).

**Effect of the Two Treatment Regimens on Serum BNP Level**

There was a significant increase in serum BNP level (pg/ml) after 2, 4, 6 and 8 cycles as compared with baseline levels in trastuzumab regimen group (p< 0.05) as shown in figure (2). In comparison between the two treatment groups, there was no significant difference in serum BNP level at baseline and after 2 treatment cycles (P > 0.05) as shown in figure (2). After 4, 6, 8 treatment cycles, serum BNP level of group II was significantly lower than that of group I (P < 0.05) as shown in figure (2).

* 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 brain natriuretic peptide (BNP) at baseline and after 4 and 8 cycles in both groups (trastuzumab therapy, n=13 and trastuzumab plus Valsartan, n=13).

**Effect of the Two Treatment Regimens on Serum MDA Level**

There was significant increase in serum MDA level (µmol/L) after 2, 4, 6 and 8 cycles as compared with baseline level in trastuzumab regimen group (p< 0.05) as shown in figure (3). In comparison between the two treatment groups, there was no significant difference (P > 0.05) in serum MDA level at baseline as shown in figure (3). After 2, 4, 6 and 8 treatment cycles, serum MDA level of group II was significantly lower than that of group I (P < 0.05) 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 4 and 8 cycles in both groups (trastuzumab therapy, n=13 and trastuzumab plus Valsartan, n=13).

**DISCUSSION**

The use of trastuzumab therapy in breast cancer with HER2 positive receptor has significantly improved response rates and increased survival in female patients with early-stage and metastatic disease. However, the unpredictably high rate of cardiotoxicity, up to a third of female patients treated with trastuzumab therapy might develop a cardiotoxicity, has generated immense concern regarding its use. In fact, the occurrence of cardiac dysfunction restricts the option of possible oncological therapies to those measured less aggressive and as a result less effective. Patients who develop impaired heart function when treated with trastuzumab might have to stop this treatment, which could influence their chances of cure.

**Effect of Trastuzumab Therapy on Clinical and Biochemical Parameters of the Present Study:**

In comparison to baseline values, there was no significant change (P> 0.05) in the echocardiographic ejection fraction after 4 treatment cycles. This finding is in agreement with that revealed by McArthur and Chia (2007) but is disagree with that revealed by Dores et al. (2013). After 8 treatment cycles, trastuzumab caused significant decrease in echocardiographic ejection fraction in comparison to baseline values (P < 0.05). This finding is in accordance with that revealed by Russo et al. (2014). The mechanism beyond this effect is that trastuzumab is positioned to block cardiomyocyteHER2 signaling. So it interfering with normal growth, repair, counteraction of excessive sympathetic tone and survival of
cardiomyocytes.\textsuperscript{21,22} Trastuzumab results in significant increase in serum BNP level after consequent treatment cycles compare with baseline level (P < 0.05). This result agree with that revealed by Periket \textit{et al.} (2006)\textsuperscript{72} and Feola \textit{et al.} (2011).\textsuperscript{24} The explanation of increase in serum BNP level may be attributed to increase load on cardiac myocyte as consequence of gene expression of renin and promoted expression of angiotensin receptor (AT1) in trastuzumab treating patient.\textsuperscript{25} BNP was observed early after receive of trastuzumab, allowing us to identify the female patients at a largest risk after the first two cycles of therapy. This result was in accordance with that revealed by Sandriet \textit{et al.} (2005)\textsuperscript{26} who advocate that BNP might be useful marker to predict and/or detect trastuzumab-induced cardiotoxicity. 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 \textit{et al.} (2014)\textsuperscript{26} and Keith \textit{et al.} (1998).\textsuperscript{27}

**Effect of Valsartan on Clinical and Biochemical Parameters**

ACE-inhibitors and ARB are standard of care in the management of patients with CHF, significantly diminishing the development of heart failure in patients with cardiac myopathy of various origins.\textsuperscript{28} In patients with doxorubicin-induced cardiac toxicity, ACE-inhibitors can improve symptoms of CHF and can avert a decline in LVEF.\textsuperscript{29,30} The ACE-inhibitors are increasingly being substituted by ARBs (e.g., Valsartan), due to the experienced intolerance to ACE-inhibitors. ARBs may induce a more entire inhibition of the RAS than ACE-inhibitors; and are unrelated to non renin-angiotensin effects such as angioedema and cough. Moreover, Valsartan has no effect on trastuzumab efficacy because it is includes in the management of trastuzumab cardiotoxicity.\textsuperscript{31}

In present study Valsartan produced a significant increase in echocardiographic ejection fraction compared with that of trastuzumab regimen group (P < 0.01). This is in consistency with that of Nakamae \textit{et al.} (2005)\textsuperscript{32} who have demonstrated that Valsartan given along with anthracycline-containing regimens prevents cardiac damage. Valsartan cause no significant effect on blood pressure and heart rate compared with control groups. Therefore, the beneficial effects of Valsartan on acute cardio toxicity are suggested to be mediated by the direct inhibition of Ang II, supporting previous animal experiments that demonstrating a key role of the renin-angiotensin system in induced cardiac damage.\textsuperscript{33} Valsartan caused highly significant decrement in serum BNP. This finding is in agreement with that revealed by Latiniet \textit{et al.} (2002)\textsuperscript{34} who have showed that Valsartan caused sustained reduction in BNP compare with control group. Bayir et al. (2009)\textsuperscript{35} reported the same result where the Valsartan prevented the acute MI-induced raise in serum BNP levels. This decrement in serum BNP level may be due to reduce load on cardiac myocyte by blocking the augmented expression of AT1 receptor. Valsartan caused significant decrease in serum MDA levels. This finding support the presence of antioxidant effect of Valsartan when administered in experimental animal model.\textsuperscript{36–38} The antioxidant effect of Valsartan attributed to block Ang II effects, which is regarded as a pro-oxidant through stimulate the generation of reactive oxygen species.\textsuperscript{29}

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