Biomarkers for evaluating response to chemotherapy in metastatic breast cancer patients

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Abstract:

**Background:** Although, different protocols of chemotherapy are recommended for the treatment of metastatic breast cancer, still response rates are variable.

**Objectives:** The aim of this study is to investigate the effects and correlation of different chemotherapy administered to metastatic breast cancer patients on serum levels of some biomarkers.

**Patients and methods:** Thirty metastatic breast cancer patients were enrolled in the study. The patients received different protocols of chemotherapy. Blood samples were taken from the patients before and after the last cycle of each protocol and from 20 healthy control and serum levels of biomarkers IL-6, leptin, CA 15-3 and p53 were estimated by Elisa.

**Results:** The mean serum levels of IL-6, leptin, CA 15-3 and p53 were significantly (P ≤ 0.01) higher in patients before and after chemotherapy compared to controls. A significant (P≤0.01) higher mean levels of IL-6, leptin and p53 was found in the patients after chemotherapy compared to before chemotherapy with the exception of mean serum levels of CA 15-3 that did not differed significantly (P≥0.174) after chemotherapy compared to before chemotherapy. A significant correlation was found between hormone status and the mean serum level of leptin and between the mean serum levels of CA15-3 and IL-6.

**Conclusion:** The results highlights that the biomarkers IL-6, leptin, CA 15-3, p53 play a role in breast cancer progression and metastasis and could be helpful in predicting and monitoring chemosensitivity to these chemotherapeutic drugs.

**Keywords:** breast cancer, chemotherapy, IL-6, leptin, CA 15-3, p53.

Introduction:

Breast cancer is the second leading cause of death from cancer in women. The standard systemic treatments for metastatic breast cancer include cytotoxic chemotherapy, targeted therapy and endocrine therapy. Various advances have been achieved in the treatment of metastatic breast cancer, but prognosis is still deficient. Increasing incidence of treatment-resistant metastatic disease, and failure of chemotherapy in eliminating cancer stem cell (CSC) population, also involved in poor prognosis in those patients. Serum biomarkers may play important roles in the diagnosis, prognosis, predicting response to specific therapies, early detection of recurrence after curative surgery, understanding mechanisms of resistance. IL-6 is one of the most potent cytokine that has a major role in promoting tumor growth through inhibiting cancer cells apoptosis and inducing tumor angiogenesis. CA 15-3, is widely used for monitoring therapy in patients with metastatic disease and elevated CA 15-3 levels found associated with poor response to chemotherapy and treatment failure. The p53 protein, facilitates antitumor drug response through a variety of key cellular functions, including cell cycle arrest, senescence, and apoptosis. However, these functions essentially cease once p53 become mutated.

In breast cancer, leptin which is secreted by adipose tissue is overexpressed and high leptin serum levels promote in situ estrogen production and directly transactivate estrogen receptor, increases expression of HER2 that promote invasion and resistance to therapy.

**Patients and Methods:**

Thirty female patients aged between 30-70 years with metastatic breast cancer who were attending Nanakaly ospital and Oncology Department in Rizgari Teaching Hospital, Kurdistan region, Erbil from December 2014 to September 2015 were enrolled in this study. Ethical committee approval from the College of Medicine and from both hospitals were obtained. The patients were instructed by the oncologist to receive chemotherapy in different protocols, 15 patients received Taxanes (Paclitaxel for 12 cycles and Docetaxel for 4 cycles), 8 patients AC (Adriamycin-Cyclophosphamide) and 7 patients Capecitabine for 4 cycles for each of them. A control group included twenty apparently healthy individuals with no history of cancer with age, BMI and sex matched with the patients group. Inclusions criteria were metastatic breast cancer (stage 4) or recurrence. Primary breast cancer, previous chemotherapy or hormonal therapy after diagnosis as metastatic breast cancer, and patients with liver, kidney impairment, and cardiac insufficiency were excluded in this study. Blood sample (5 ml) was withdrawn from each patient before administration of chemotherapy and after the last cycle of chemotherapy. Serum was separated and introduced...
into small vacutainers and stored at -70°C for biomarkers assay IL-6, Leptin, CA15-3, and P53 ELISA. The biomarkers used were Leptin ELISA kit (Dbc, Canada), CA15-3 ELISA kit (Cobas, USA), Human IL-6 ELISA kit (AviBion, Finland), p53 ELISA kit (IBL, Germany).

**Statistical analysis:**
Data were analyzed statistically using SPSS Version 20.0 for Windows. All the data were expressed as Mean ± SD. Comparisons between groups was determined by Duncan test. Pearson correlation was used to find the correlation between different parameters. Mann-Whitney test was used to compare differences between two independent groups when the dependent variable is not normally distributed data. P value of 0.05 or less was considered statistically significant.

**Results:**
The age of patients ranged from 30-70 years with a mean of 48.8 ± 10.25 years and a mean body mass index (BMI) of 32.7 ± 8.74 (Table1). ER+/PR+ was expressed in 66.7% patients and 33.3% patients expressed ER-/PR- while HER2+ and HER2- was expressed in 40% and 60% of patients respectively and 10% patients expressed triple negative breast cancer (TNBC) as shown in table (1). Concerning chemotherapy: 50% of the patients received taxanes (paclitaxel and docetaxel), 26.7% received AC and 23.3% patients received capecitabine (Table 1). Patients with had metastasis to one site either bone, or liver or lung constituted 73.3% and 26.7% had metastasis to more than one site (Figure 1).

**Table (1): Demographic characteristics of the patients (n=30) enrolled in the study**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Healthy Control</th>
<th>Before Chemo-therapy</th>
<th>After Chemo-therapy</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>3.83±0.1^B</td>
<td>9.87±2.28</td>
<td>22.65±4.97</td>
<td>0.0001</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>13.22±3.56</td>
<td>40.45±5.87</td>
<td>58.14±6.23</td>
<td>0.0001</td>
</tr>
<tr>
<td>CA15-3 (U/ml)</td>
<td>17.42±1.16</td>
<td>148.36±29.68</td>
<td>125.92±29.40</td>
<td>0.174</td>
</tr>
<tr>
<td>P53 (U/ml)</td>
<td>0.017±0.01</td>
<td>0.77±0.05</td>
<td>1.71±0.027</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*P value ≤ 0.05 was considered statistically significant. Different latter indicate significant differences between groups.*

No significant differences was found between mean serum levels of L-6, leptin, CA15-3 and p53, whereas, a significant difference (P ≤ 0.05) between serum level of leptin in patients with ER+/PR+ and ER-/PR- (Table 3).
Biomarkers for evaluating response to chemotherapy in metastatic breast cancer patients

Nidhal A.K. Ali

Despite improvements in treatment options, breast cancer remains one of the leading causes of mortality in women. The average BMI (32.7±8.74) of the patients in this study indicates the patients are overweight or obese according to the WHO recommended BMI cut-off points for overweight and obesity as ≥25 kg/m² and ≥30 kg/m² respectively. Evidences exist that breast cancer risk is positively associated with BMI in postmenopausal women and they have a 25% increase in the risk of developing breast cancer. In addition, obese women with breast cancer have more chance of treatment failure when compared to lean women. Endocrine changes that contributes to increased risk of developing breast cancer in obese postmenopausal women is related to increased activity of aromatase enzyme that results in increased formation of estradiol. The lack of significant (P ≥ 0.311) correlation between mean p53 serum level and HER2 overexpression in this study has also been reported with taxanes as high expression of HER2 and p53 found associated with advanced stage of the cancer and with poor survival in breast cancer 14 and that HER2 signaling cascade influences activity of several chemotherapy by altering cell survival, apoptosis, drug efflux, and drug metabolism. The significantly (P ≤ 0.05) higher serum IL-6 levels in the patients in the current study after receiving chemotherapy as compared to control and before administration of chemotherapy, has been demonstrated that increased IL-6 serum levels in tumor microenvironment protect cancer cells against chemotherapy, increases the expression of several survival proteins, including Bcl-2 XL and Bcl-2XL, and confers resistance to apoptosis induced by doxorubicin, paclitaxel, and trastuzumab and associate with worse survival in patients with metastatic breast cancer and correlate with the extent of disease migration 15,16,17. In breast cancer, doxorubicin found to induce inflammation, NF-κB, VEGF, and increased levels of inflammatory markers such as IL-1β and IL-6, promote bone and lung metastasis through inducing epithelial mesenchymal transition (EMT) mediated by production of TGF-β as well as tumor cell migration and invasion 18. Data showed that multidrug resistant cells have a stronger immune-suppressive attitude than chemosensitive cells, due to the constitutive activation of the JAK/STAT3/indoleamine 2, 3-dioxygenase 1 axis, thus resulting in chemoresistance to doxorubicin and paclitaxel. This evidence may also reflect the significantly (P ≤ 0.05) higher serum IL-6 levels in the patients in this study after receiving chemotherapy as compared to those before chemotherapy. Numerous in vitro and in vivo studies reported that IL-6 is capable of modulating the expression and function of different drug transporters that increases their resistance to chemotherapeutic agents including doxorubicin, and paclitaxel. This fact might explain the significantly (P ≤ 0.0001) higher IL-6 serum levels after administration of chemotherapy compared to serum levels before chemotherapy.

Table (3): Mean serum levels of IL-6, Leptin, CA15-3 and p53 in HR+ patients and HR- patients (n= 30) after administration of chemotherapy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ER+/PR+ patients</th>
<th>ER-/PR- patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>26.22±5.38</td>
<td>15.51±4.63</td>
<td>0.209</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>47.82±6.59</td>
<td>78.79±11.00</td>
<td>0.016</td>
</tr>
<tr>
<td>CA15-3 (U/ml)</td>
<td>137.01±41.15</td>
<td>103.74±33.56</td>
<td>0.602</td>
</tr>
<tr>
<td>p53 (U/ml)</td>
<td>1.89±0.39</td>
<td>1.34±0.26</td>
<td>0.354</td>
</tr>
</tbody>
</table>

P value ≤ 0.05 was considered statistically significant.

There was no significant differences in the mean serum IL-6, Leptin, CA15-3 and p53 levels in patients with 1 metastatic site and > 1 metastatic site patients (Table 4) and after administration of different chemotherapy protocols (Table 5). A statistical significant (P ≤ 0.039) correlation (r = 0.392) was found only between IL-6 and CA15-3 serum level after administration of different chemotherapy protocols (Figure 2).

Table (4): Mean serum IL-6, Leptin, CA15-3 and p53 level in patients with 1 metastatic site and > 1 metastatic site patients (n= 30) after administration of different chemotherapy protocols.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 metastatic site patients</th>
<th>&gt; 1 metastatic site patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>19.32±3.88</td>
<td>31.83±10.2</td>
<td>0.167</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>60.0±7.18</td>
<td>53.04±13.16</td>
<td>0.630</td>
</tr>
<tr>
<td>CA15-3 (U/ml)</td>
<td>115.69±33.09</td>
<td>154.07±56.13</td>
<td>0.573</td>
</tr>
<tr>
<td>p53 (U/ml)</td>
<td>1.89±0.35</td>
<td>1.2±0.3</td>
<td>0.276</td>
</tr>
</tbody>
</table>

P value ≤ 0.05 was considered statistically significant.

Table (5). Mean serum level of IL-6, Leptin, CA15-3 and p53, in patients (n= 30) after administration of taxanes, AC, and capecitabine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients on Taxanes</th>
<th>Patients on AC</th>
<th>Patients on capecitabine</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>18.51±0.5</td>
<td>23.4±5.83</td>
<td>30.69±9.28</td>
<td>0.485</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>53.31±7.0</td>
<td>58.59±15.34</td>
<td>68±14.17</td>
<td>0.658</td>
</tr>
<tr>
<td>CA15-3 (U/ml)</td>
<td>93.73±36</td>
<td>159.8±65.29</td>
<td>156.08±68.84</td>
<td>0.583</td>
</tr>
<tr>
<td>p53 (U/ml)</td>
<td>1.97±0.4</td>
<td>1.49±0.55</td>
<td>1.39±0.3</td>
<td>0.641</td>
</tr>
</tbody>
</table>

Figure (2): Correlation between serum levels of IL-6 and CA 15-3 in the metastatic breast cancer patients (n=30).

Discussion:
assuming the tumor cells in the patients might have developed resistance to these chemotherapy. Other reason for the significantly higher serum IL-6 levels in the patients in the current study after receiving chemotherapy besides implication of resistance could be attributed to the role of cancer stem cells (CSC) which has been shown to increase resistance to radiation, chemotherapy such as docetaxel, trastuzumab, and tamoxifen. The mean serum levels of leptin that revealed a significant elevation (P ≤ 0.05) in breast cancer patients before treatment compared to controls is in agreement with the findings of other studies that demonstrated also correlation of leptin with increased risk of breast cancer and associated with distant metastasis. Breast tumor cells found to acquire high ObR expression under the adiposity enriched environment and generate a population with enhanced CSC properties, tumorogenic capacity involved in the stabilization of CSC phenotype. The role of leptin in breast cancer is crucial as interplay in mediating tumor-stroma interaction and influencing EMT-linked mechanisms; which is an early step during tumor metastasis that sustain breast cancer growth and progression. This evidence, might explain the significantly (P≤0.05) higher levels of leptin in the patients in this study compared to controls. It has been reported that ERα plays an important role in chemoresistance to paclitaxel hindering the cytotoxic effects; this might explain the significantly (P≤0.05) higher serum levels of leptin in the patients after chemotherapy when compared with the levels before chemotherapy administration. Evidence has indicated that leptin and estrogen might cooperate in maintaining estrogen-dependent breast cancer growth. These statements might explain the significant (P≤0.05) correlation between leptin and HR+ shown in this study. Other data showed that leptin promote cellular proliferation not only in ER+ but also in ER- breast cancer cell lines. This evidence was based on a strong correlation between leptin signaling and expression of an oncogenic enzyme; Sphingosine kinase 1 (SK1) in breast tumors, which have a physiological significance in obesity driven ER- breast cancer. This finding most likely explain the significant correlation (P≤0.05) between leptin levels and HR- status in the patients in this study. There have been lack of reports investigating prognostic effects of leptin in metastatic breast cancer patients after chemotherapy that support the potential use of leptin-signaling inhibition as a novel treatment for ER+ and ER- breast cancer. The usefulness of measuring CA 15-3 levels in patients with breast cancer remains controversial. The significant (P≤0.05) higher mean serum levels of CA 15-3 in breast cancer patients pre and post treatment respectively compared to normal healthy controls indicate it is overexpressed during metastasis and is useful as a tumor marker for metastatic breast cancer patients follow up since elevation of CA 15-3 serum levels greater than the cutoff 30 U/ml shown correlated with tumor progression and malignancy. Monitoring CA 15-3 levels is recommended to follow up patients receiving chemotherapeutic or anti-hormonal therapy and increased CA 15-3 serum levels was considered a marker reflect signal of the presence of tumor and metastasis. Indeed in this study, the patients had metastasis to different sites (bones, liver, and lung) therefore, the significant elevation in CA 15-3 serum levels before chemotherapy compared to controls probably coincide with these observations. The significant (P ≤ 0.05) correlation that was found between serum levels of CA 15-3 and IL6 in the patients after chemotherapy most likely express the insufficient control of cancer by the chemotherapy and reflect possibility of chemotherapy resistance since STAT3 activation is associated with tumor survival and therapeutic resistance along with pro-metastatic behavior reported for paclitaxel and illustrate metastasis particularly 30% of the studied patients had bone metastasis. The mean p53 serum levels that was significantly (P≤0.05) higher in the patients in this study before and after receiving chemotherapy respectively as compared to controls indicate that this tumor suppressor protein is upregulated in these patients. Mutation of p53 have been described in breast cancer and observed in 30% of breast carcinomas and found to contribute to tumorigenesis and confer aggressive tumor behavior that is not seen in p53-wild type. Under normal conditions, the wild-type p53 protein is normally undetectable or expressed at low levels in all human cells because it is rapidly degraded with short half-life (~20 minutes) except under conditions of cellular stress. In contrast, mutant proteins is often associated with the production of a stable protein, which increases the half-life that is readily detectable. On this ground, in the present study, the mean serum level of p53 that was detected in the apparently healthy control was considered the cutoff value for normal p53, whereas; the levels in the patients before and after chemotherapy respectively are considered indicative for the mutant p53. A possible explanation for the significant (P ≤ 0.05) higher mean serum levels of p53 in the patients after administration of chemotherapy compared to those before chemotherapy, could be related collectively to; p53 overexpression, or chemoresistance to taxanes, doxorubicin, and tamoxifen; that is administered along with chemotherapy to the patients. Since the correlations between mean serum levels of p53 in patients in the current study with HR+ and HR- was not significantly different, thus, it is most likely expected that these patients are resistant to the effect of administered chemotherapeutic agents. Thus, effective treatment for these tumors expressing ER, may require the concomitant complete inhibition of ER signaling with drugs such as fulvestrant, a drug that leads to the down-regulation and degradation of the ER together with treatments that promote p53-mediated apoptosis. Recent studies have demonstrated that p53 directly activate Toll-like receptor 4 (TLR4) and in p53 mutant
breast cancer cells, TLR4 activation alters the balance of progrowth and antigrowth cytokines in the extracellular microenvironment, ultimately resulting in increased proliferation and growth and promote chemoresistance with taxanes chemotherapy 33,34. Furthermore, paclitaxel shown to induce activation of TLR4-NF-κB pathway leading to upregulation of prosurvival genes Bcl-2, Bcl-xl proteins besides mutant p53, enhances the survival of cancer cells promote chronic inflammation, peripheral neuropathy, angiogenesis, and recovery of damaged cells 34,35. Collectively, these results demonstrate that paclitaxel not only kills tumor cells but meanwhile it enhances their survival by activating TLR4 pathway. Therefore; a possible explanation for the significant (P ≤ 0.05) increase in p53 levels shown in patients in this study after chemotherapy compared to control apparently healthy and to those before receiving chemotherapy is most likely attributed to either; the increase in mutant p53 levels as consequence to chemotherapy induced activation of TLR4 by the taxanes and doxorubicin that subsequently leads to activation of transcription factor NF-κB, a prosurvival factor and causes upregulation of p53 or to chemoresistance induced by MDR and metastasis 36. The ability of paclitaxel in increasing tumor cell invasion is of particular significance and a variety of human cancer cell lines, showed that paclitaxel is one of the most potent inducers of invadopodia, which is a cell protrusion required for invasion 37. Besides these, hypoxia also shown to induce mutant p53 as well as resistance to paclitaxel through reactive oxidative species (ROS)-mediated HIF-1α stabilization 38. The central role of p53 in the control of apoptosis, senescence and cell cycle arrest, remains a puzzle for its possible role in predicting response to chemotherapy in breast cancer patients as there are unconvincing response for tumors with functional or mutant p53. Attempts are required to be directed toward personalized medical treatment because of genomic variation including polymorphisms, gene expression levels, and epigenetic changes in both the host and tumor tissue and another point that should not be forgotten is to weigh the benefits over the risk of cancer 39, 40. The non-significant differences in the mean serum levels of the biomarkers, in patients with 1 metastatic site and > 1 metastatic site and after administration of different chemotherapy protocols obviously demonstrate insufficient control by different chemotherapy in the studied patients.

**Conclusion:**

There remain gaps in our knowledge that demand further research, for understanding the factors contributing to cancer occurrence, metastasis, and drug resistance. Therefore, investigating the potential role of tumor markers is of high importance to fill the gaps between basic research and clinical application.

**Authors Contributions:**

Nidhal AK Mohammed Ali: Research designer, arrangement and follow up collection, analysis of samples and data and writing the manuscript.

Rehab Mohammed Younis: Collecting samples, analysis of samples and writing the manuscript.

Jangi S. Salai: Facilitate collection of blood samples in the hospital.

**References:**


