Bone morphogenetic proteins (BMP-2, BMP-7, and BMP-12) and chitotriosidase as novel markers in detection and staging of breast cancer in Iraqi women

Zahraa M. Ali* Shatha H. Ali* and Furat Y. Mohsen**

*Department of Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad, Iraq. **Ministry of Health and Environment, Medical city, Oncology Teaching Hospital, Baghdad, Iraq

1Corresponding author: Zahraa M. Ali (mail: obaydizahra@yahoo.com) Phone: +964-7901666543

Abstract
Breast cancer (BC) is the most commonly diagnosed cancer in women, bone morphogenetic protein (BMPs) are highly correlated to various aspects of carcinogenesis, whereas chitotriosidase is chitinase that mediates several processes and synergistic effects with proteases and other enzymes to kill different types of pathogens and cancer cells. The present study was aimed to assess the validity of the measurement of several BMP as tumor markers aid in detection or staging of breast cancer. These include three types of growth factors: (BMP-2, BMP-7 & BMP-12) besides chitotriosidase enzyme levels in Serum. This study included 66 women with breast cancer. Those patients were categorized into three groups (22 in each group) according to disease stage, in addition to group 4 and chitotriosidase were estimated quantitatively using ELISA kits. Data analysis revealed that although serum levels of studied BMPs were not significantly different among studied groups, serum BMP-7 of patients at stage III presented with significantly higher levels than the controls, while serum BMP-12 levels were lowered significantly at stages I & II breast cancer patients as compared to the controls. Furthermore, elevated serum chitotriosidase levels were only detected at stage III levels only as compared to the controls. Thus both serum BMP-7 and chitotriosidase can be recommended as markers for prognosis of BC to advanced stages (stage III), while serum BMP-12 is recognized as a marker to identify patients with breast cancer at early stages (I & II).

Keywords: Breast cancer, chitotriosidase enzyme, bone morphogenetic proteins, serum tumor marker

Introduction
Breast cancer (BC) is the most commonly diagnosed cancer in women and about one in 4 of all new cancer cases diagnosed in women worldwide are breast cancer, it is also the leading cause of cancer death in women [1]. The updated recommendations of the American Society of Clinical Oncology (ASCO) included the use of tumor markers in prevention, screening, treatment, and surveillance of breast cancer. According to this recommendation, there were 13 categories of tumor markers were considered. The tumor markers that were recommended for practical use include CA 15-3, CA 27.29, Estrogen receptor (ER), Progesterone receptor (PR), Human epidermal growth factor receptor 2 (HER2), Carcinoembryonic antigen (CEA), Plasminogen activator inhibitor 1 (PAI-1) Urokinase plasminogen activator (uPA), and multiparameter assays for gene expression [2]. Bone morphogenetic proteins (BMP) are growth factors; they control various cellular processes, such as proliferation, differentiation, apoptosis, and migration by regulating target gene transcription [3]. Furthermore, BMPs are highly related to different aspects of carcinogenesis, such as angiogenesis, epithelial-mesenchymal transition (EMT), and cancer stem cells [4, 5]. There is increasing evidence indicate that BMP proteins and their signaling components can be regarded as novel biomarkers for cancer treatment and can offer significant therapeutic implications even though the expression of specific BMPs still controversial [6]. The interactions between BMPs and their antagonists or receptors significantly support the identification of the aggressiveness of primary tumors and establish a mechanism for cancer cell metastasis [6]. BMP-2 reduced cancer cell proliferation and it may be down-regulated in breast cancer. BMP-2 can also promote oncogenic behavior by affecting apoptosis, migration, invasion, and angiogenesis. While BMP7 is widely expressed in breast cancer and is associated with early bone metastasis [7]. But, the BMP-12 role in cancer remains unknown, which shows decreased expression in human breast cancer as compared to normal breast tissue. Such a reduction in BMP-12 expression may associate with disease progression and poor prognosis. It had been suggested that BMP-12 may act as a putative inhibitory factor in breast cancer [8].

©Annals of Tropical Medicine & Public Health S262
Whereas chitotriosidase enzyme (CHIT1, EC: 3.2.1.14), is chitinase belonging to the family of 18 glycosyl hydrolases [9]. In man, chitotriosidase is mainly expressed by different lineages of activated blood and tissue macrophages [10] and also to a lesser extent by polymorphonuclear leucocytes [11]. Many processes illustrated the immunomodulatory effects of chitotriosidase including chitin recognition, antigen presentation, induction of cell-mediated immunity and synergistic effects with proteases, and other enzymes to kill different types of pathogens and cancer cells [10, 12-15]. Meanwhile, chitinases and chi-lectins could play a detrimental role in the development of human cancer, especially CHI3L1 (YKL-40) and has been associated with increased tumor angiogenesis and bad prognosis in many of human neoplasms such as breast, lung, and cervical cancers (16-18), also it is believed that chitinases have some anticancer cell activities [19].

The goal of this study was to assess the validity of several serum tumor biomarkers to aid in the detection and staging of breast cancer, as blood markers are easily obtained and measured than tissue samples. These studied markers include BMP-2, BMP-7, BMP-12, and chitotriosidase in addition to alkaline phosphatase activity as a routinely obtained measure that would give additional information on disease prognosis.

Materials and methods
This case-controlled study was conducted at The Oncology Teaching Hospital / Medical City from October/2018 to February 2019 and included the following groups: 1. Disease Study Group: This group included 66 patients (females) with age range of 32-68 years, mean age ± SE was 48.24±1.8 years, with histologically confirmed breast cancer by a specialized oncologist and, none of the patients had any other malignancy. Only newly diagnosed patients with no prior chemo-, radiation- nor hormonal therapy were enrolled in the study. Those patients were categorized into three subgroups (22 in each group) depending on the disease stage that was determined by the TNM staging system [20]. Group 1 includes patients with stage I disease with age range of 32-67 years, mean age ±SE was (51.5±2.3) years. Group 2 includes a patient with stage II disease with age range of 34-68 years, mean age ±SE was (47.6±1.87) years. Group 3 includes patients with Stage III of disease with age range of 32-65 years, mean age ±SE was (45.56 ±1.75) years. In addition to the control group, (Group 4): include 22 apparently healthy females with age range of 31-60 years, mean age ±SD was (44.3±1.5) years, these control subjects were matched for age, sex with the patient study groups. The study was authorized by the Local Research Ethics Committee and all subjects were provided with written informed consent to participate in this study, some of the estimated biomarkers for patients diagnosed to have breast cancer to be included in the study are illustrated in table 1. Serum levels of BMP2, BMP, BMP12, and chitotriosidase were estimated quantitatively using specific ELISA kits[21]. In addition to serum levels of ALP [22], urea [23], creatinine [24] which were measured using Dimension clinical chemistry system, Flex reagent cartridge. Analysis of data was carried out using the statistical package of SPSS-23 (Statistical Packages for Social Sciences-version 23). Data were presented in simple measures of percentage, mean, standard error, and range. Statistical significance was considered whenever the P-value was equal or less than 0.05.

Table -1 Basic characteristics of subjects included in the study

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Total patient (n=66)</th>
<th>Stage I (n=22)</th>
<th>Stage II (n=22)</th>
<th>Stage III (n=22)</th>
<th>Control (n=22)</th>
<th>p-value t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean age (year)</td>
<td>48.4±1.8</td>
<td>51.5±2.3</td>
<td>47.6±1.8</td>
<td>45.5±1.7</td>
<td>44.3±1.5</td>
<td>0.064</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>28.8±0.5</td>
<td>28.4±0.4</td>
<td>28.9±0.4</td>
<td>29.1±0.7</td>
<td>26.9±0.8</td>
<td>0.041*</td>
</tr>
<tr>
<td></td>
<td>ER positive</td>
<td>43</td>
<td>10</td>
<td>16</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PR positive</td>
<td>44</td>
<td>12</td>
<td>16</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Her positive</td>
<td>28</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IDC</td>
<td>45</td>
<td>16</td>
<td>15</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DCIS&amp;IDC</td>
<td>15</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ILC</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ILC&amp;IDC</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemoglobin(g/dl)</td>
<td>12.19±0.16</td>
<td>12.2±0.28</td>
<td>12.4±0.37</td>
<td>11.9±0.18</td>
<td>12.7±0.13</td>
<td>0.009*</td>
</tr>
</tbody>
</table>
Results

Figure-1 showed that serum BMP-2 levels of different patient groups were not significantly different from that of the control group, with no significant differences among patient groups. Only BMP-7 levels of stage III patient group showed a significant increase over that of the control group with no significant differences between patient groups (figure-2), while serum BMP-12 levels of group 1 and group 2 were significantly lower than that of the control group, as shown in (figure -3). Meanwhile, serum BMP-7 values were significantly correlated with BMP-12 levels at stage I patients ($r=0.565,p=0.006$). On the other hand, all groups of patients showed higher serum chitotriosidase levels than that of the control group and only that of the stage III patient group was significantly elevated, with no significant differences between patient groups (Figure-4).

Among other studied biomarkers in serum activity of ALP where the levels of all patient groups were significantly elevated as compared to that of control with significant differences between that of stage I and stage III as well as between stage II and stage III groups (table-2). Furthermore, BMP-12 levels were significantly correlated with ALP through all stages of the disease ($r=0.3$, $p=0.014$) as presented in table - 3. However, there was no significant variation among different stages of the disease, nor with that of the control for serum urea and creatinine levels (table-2).

Table-2: Serum levels of some parameters

<table>
<thead>
<tr>
<th>group Parameter</th>
<th>Total patient n=66</th>
<th>Stage I (n=22)</th>
<th>Stage II (n=22)</th>
<th>Stage III (n=22)</th>
<th>Control (n=22)</th>
<th>P value t-test</th>
<th>groups</th>
<th>P value ANOVA</th>
<th>groups</th>
<th>P value ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP IU/l</td>
<td>94.84±4.8</td>
<td>84.9±4.7</td>
<td>87.5±6.9</td>
<td>112.09±11.08</td>
<td>62.4±3.4</td>
<td>0.000*</td>
<td>1-1-31-4</td>
<td>0.802</td>
<td>0.00*</td>
<td>0.02*</td>
</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.749±0.016</td>
<td>0.76±0.02</td>
<td>0.73±0.02</td>
<td>0.74±0.03</td>
<td>0.7±0.02</td>
<td>0.189</td>
<td>1-2</td>
<td>0.349</td>
<td>0.586</td>
<td>0.12</td>
</tr>
<tr>
<td>Urea mg/dl</td>
<td>27.6±1.19</td>
<td>26.4±1.7</td>
<td>29.2±2.9</td>
<td>27.3±1.1</td>
<td>22.09±1.4</td>
<td>0.015*</td>
<td>1-2</td>
<td>0.308</td>
<td>0.742</td>
<td>0.12</td>
</tr>
</tbody>
</table>

* Significantly different from compared group ($p<0.05$).
Figure 1: Serum level of BMP-2 (pg/ml) of studied groups

Figure 2: Serum level of BMP-7 (pg/ml) of studied groups

*=significantly different from control (p˂0.05)
Discussion

BMPs are described both as stimulators and inhibitor in different cancers; thus, it is difficult to define BMPs as oncogenes or anti-oncogenes. Many pieces of evidence indicated that their effects of signaling on tumor progress depend on the cell types and the tumor microenvironment [25-39]. In breast cancer cell lines or tumor tissues, BMP-2 expression is often decreased [26, 7]. A study reported that BMP-2 led to G1 arrest by inducing the expression of p21 to inhibit the proliferation of breast cancer cells [27, 28]. Other lines of evidence showed that BMP-2 inhibits apoptosis [29, 30] rather than inhibiting the proliferation of breast cancer cells [31]. However, the BMP-2 effect on breast cancer is complicated and unclear, and further studies are required. This study revealed no significant differences between serum levels of BMP-2 among different patients groups neither when compared to healthy control group (figure 1), this may result from several factors, first is the patient number in each group, the effect of BMP-2 on breast cancer is complicated and there is overlapped role for most types of BMPs in different cancer types not only in breast cancer.

BMP7 has been proposed as a marker of differentiation in normal and breast cancer cells [32-37], even that, it’s a role in breast cancer progression remain largely elusive [32, 33]. BMP7 has two opposing effects on...
cell proliferation and the net effect depends on the cell type and differentiation state of the cells [34-35,38]. The results of our study indicate a steady elevation of serum level of BMP7 through the BC stages (stage I, II and III) as compared to control group, although such elevation was not significant except for BMP-7 level of stage III patient group who show significant difference from that of control group, also there was no significant difference among the patients groups (Figure-2).

The correlation between decreased BMP-12 expressions with poor prognosis indicates that BMP-it 12 may be an inhibitory factor during the disease progression and may have potential prognostic implications in breast cancer [8]. This study indicated that the serum levels of BMP-12 decrease at all stages of d, diseases when compared to that of healthy controls but patients at stage III of the disease do not reach significant levels. The his may result from interfering factors ta hat may be affected on serum BMP-12 levels, as these patients have the higher ratio of family history (81.8% vs 18.18% and 22.7% for patients at stage I and stage II respectively ) (Table-1), and this may indicate to the role of genetic factors. Also, a higher percentage of anemic patients was in patients with stage III of disease as (54.5% vs 18.18 &22.7% for stage I and stage II patients respectively) (table -1), and this may indicate the importance of examining of association between factors affecting BMP-12 serum levels and that controlling iron metabolism that require to be examined in further studies, also there is another study still in press that study the relation between iron regulatory proteins (hepcidin and ferroportin) and BMP-12 serum level in BC patients. Another factor may be related the age distribution of patients groups, as the mean age of patients at stage III of disease was lower than that of patients at stages I and II of disease (46.2 vs 48.6 &51.5 for stage I and stage II respectively) (table -1), which may indicate to the effect of age on BMP-12 expression and its serum levels.

Furthermore, chitotriosidase activity is a sensitive biomarker of macrophage activation, so it is currently more commonly used in clinical practice for the evaluation of the status and response to treatment of inflammatory based diseases in which there is a significant role for macrophages [36,38]. In this study, the increase in serum levels of chitotriosidase in all stages of the disease to reach a significant level at stage III agree with the role of this enzyme in the cancer process as a macrophage activation marker, and this role comes as a result of inflammation in breast cancer.

Our conclusion for this study referred to the usefulness of measuring BMP-7, BMP-12, and chitotriosidase in breast cancer patients as both serum BMP-7 and chitotriosidase can be recommended as markers for prognosis of BC to advanced stages (stage III), while serum BMP-12 is recognized as a marker to identify patients with breast cancer at early stages (I &II) as its levels decreased significantly when compared to healthy women. Hence, we recommend a larger scale study to confirm these findings to be considered in detecting and staging breast cancer.

Acknowledgements
I would like to thank all employees in The Oncology Teaching Center (Medical city/Baghdad), doctors, nurses, lab staff and finally all the breast cancer patients and peoples for helping me to achieve this work.

References


