

Serum Y-Box Binding Protein 1 (YBX-1) and Interleukin 6 (IL-6) Are Associated with Metastasis in Breast Cancer Patients

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Abstract

Objectives: The aim of this study was to assess the levels of Y-box binding protein 1 (YBX-1) and interleukin 6 (IL-6) in the sera of metastatic and non-metastatic breast cancer patients (BC), investigate their clinicopathological significance and to analyze their potential use as biomarkers of breast cancer metastasis. **Methods:** The study included ninety subjects sub-grouped equally into metastatic BC, non-metastatic BC and healthy volunteers. Serum YBX-1 and IL-6 were quantified using ELISA technique while CA 15-3 was quantified using IRMA kit. Clinical data were collected from patients' records. **Results:** YBX-1 ($p < 0.001$), IL-6 ($p < 0.001$) and CA15-3 ($p = 0.017, 0.001$) were significantly elevated in metastatic and non-metastatic BC patients compared to healthy controls, however, only YBX-1 ($p < 0.001$) and IL-6 ($p < 0.001$) showed a significant difference with cancer metastasis. Generally, YBX-1 and IL-6 were correlated with worse histological grade and late clinical stage in breast cancer patients and they were also associated with axillary lymph nodes involvement and positive vascular invasion in metastatic BC patients. Serum YBX-1 and IL-6 levels were positively correlated to each other ($r_s = 0.615, p < 0.001$) and they showed high sensitivity and specificity compared to CA 15-3 ($p < 0.001$ and $p = 0.004$ for YBX-1 and IL-6 respectively) for predicting cancer metastasis. **Conclusions:** Serum YBX-1 and IL-6 are potential biomarkers of breast cancer patients with significant correlation with bad clinicopathological characteristics. Serum YBX-1 and IL-6 have superior sensitivity and specificity compared to CA15-3 and can serve as potential follow up and prognostic markers.

Keywords

Breast Cancer, Metastasis, Y-Box Binding Protein 1, Interleukin-6, Biomarker

1. Introduction

Breast cancer (BC) is the most commonly diagnosed female-associated malignancy with an incidence rate of more than 2 million in 2018. Breast cancer is also the leading cause of cancer death in women which is mainly attributed to cancer metastasis [1]. Good management of metastatic BC requires reliable biomarkers that aid in evaluation of tumor burden and determine response to treatment protocols. Y-Box binding protein 1 (YBX-1) is an evolutionary conserved cold shock pleotropic protein that belongs to the Y-box transcription factors [2]. These classes of proteins are identified by their ability to regulate the gene expression by binding to the Y-box promoter region [3]. Many reports have highlighted the role of YBX-1 in the regulation of vital processes including DNA replication [4], repair [5] and transcription [6] as well as mRNA splicing [7] and translation [8]. YBX-1 was also found to be dysregulated in many types of cancer including cervical [9], ovarian [10], gastric [11] and colorectal [12]. Strong evidence has supported the role of YBX-1 in carcinogenesis related events including uncontrolled cell proliferation, genomic instability, cell immortality and induction of angiogenesis [13] [14]. In breast cancer (BC), YBX-1 is linked to drug resistance and promotion of tumor invasion and metastasis through enhanced epithelial-to-mesenchymal transition (EMT) [15] [16].

Tumor microenvironment is usually characterized by an abnormal release of proinflammatory cytokines, which plays an important role in cancer metastasis [17]. Of the important cytokines involved in this process, interleukin 6 (IL-6) have been linked with induction of drug resistance, stem cell like characteristics and EMT induced metastasis in breast cancer [18] [19] [20]. Interestingly, few number of breast cancer studies have suggested that YBX-1 interacts with IL-6 creating a positive feed-forward loop driving EMT-like metastatic properties during cancer progression [21].

Despite the strong evidence of YBX-1 role in breast cancer invasion and metastasis, it's clinical significance and association with clinicopathological characteristics is not been thoroughly investigated yet. Herein, we conducted this study to assess the serum levels of YBX-1, IL-6 as well as the traditional follow-up marker, cancer antigen 15-3 (CA 15-3) in metastatic and non-metastatic breast cancer patients. Furthermore, we analyzed the association and correlation of all markers with patients' clinicopathological characteristics.

2. Materials and Methods

2.1. Study Population and Design

The current study included ninety subjects. Sixty female BC patients aged between 30 and 73 years from those referred to Cancer Management and Research Department, Medical Research Institute, University of Alexandria, Egypt from 2012 to 2018. Enrolled patients were selected from those who had no history of any type of malignancy and didn't receive neoadjuvant treatment then they were

subdivided into metastatic and non-metastatic groups ($n = 30$ each). The study also included thirty healthy female volunteers with matched age.

Cancer treatment was received according to physician description. The majority of patients (75%) received postoperative radiotherapy followed by 6 cycles of FAC (5-Fluorouracil, Adriamycin and Cyclophosphamide). While for the rest of patients, radiotherapy was supplied after finishing the last chemotherapy cycle. Demographic and clinicopathological data were collected from pathology reports and patients' follow-up records which included: age, family history, menopausal status, hormone receptors' status (semi-quantified by immunohistochemistry), histological grade, vascular invasion, tumor size, lymph nodes involvement, clinical stage, and site of metastasis (where applicable).

Ethical approvals for subjects' recruitment were obtained from the local Ethics Committee of Medical Research Institute, University of Alexandria and informed consents were obtained from all subjects. The study was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines.

2.2. Quantitative Determination of YBX-1, IL-6 and CA 15-3 Serum Levels

A peripheral blood sample was collected from all subjects in plain vacutainer tubes. Separation of serum was done by centrifugation at 2000 g for 10 min at 4°C. Serum was then aliquoted and stored at -80°C till use and repeated freeze and thaw cycles were avoided. Enzyme-linked immunosorbent assay (ELISA) kits were used to quantify both YBX-1 (Cloud-Clone Corp., USA) and IL-6 (Invitrogen, Thermo. Fisher Scientific, USA) while immunoradiometric assay (IRMA) was used to quantify CA 15-3 (Diasource Immunoassays S.A., Belgium) according to manufacturers' instructions. For ELISA kits, absorbance at 450 nm was measured in an InfiniteM200 Plate Reader (Tecan). For CA 15-3 IRMA kit, radioactivity was measured using Wizard2 2470 automatic gamma counter (Perkin Elmer Inc., USA).

2.3. Statistical Analysis

Data were analyzed using SPSS software package version 20.0 (IBM Corporation, Chicago, Illinois, USA). Quantitative data were described using mean \pm standard error mean. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test. Mann-Whitney test was used to compare between two studied groups, and Kruskal Wallis test was used to compare between more than two groups. Spearman correlation test was used to study the correlation between YBX-1, IL-6 and CA 15.3 serum concentrations and the clinicopathological parameters, also used to correlate YBX-1 and IL-6 concentrations. Receiver operating characteristic (ROC) curve was done to interpret the sensitivity and specificity of YBX-1 and IL-6 against CA 15-3 for predicting BC metastasis. At all statistical analyses, p value was considered significant at ≤ 0.05 .

3. Results

3.1. Patients' Characteristics

The clinicopathological characteristics of all enrolled BC patients are presented in **Table 1**. The majority of enrolled patients were post-menopausal with negative family history of breast cancer. All patients were diagnosed with invasive ductal carcinoma and underwent modified radical mastectomy. Two-thirds of patients had a tumor size less than 5 cm and their lymph node involvement ranged from N0 to N3 with percentages 23.3%, 41.7%, 5%, 30% respectively. Regarding hormone receptor status, most patients had varying degrees of ER α /PR positive expression though almost 60% lacked Her2/neu receptor expression. The majority of patients had histological grade II whilst 50% of patients presented with clinical stage IV as well as metastasis. The site of metastasis varied between bone (30%), lung (26.7%), liver (20%) and other distant sites. Regarding hormonereceptor expression.

3.2. Serum Levels of YBX-1, IL-6 and CA 15-3 in Metastatic BC, Non-Metastatic BC and Healthy Controls

Data presented in **Figure 1** shows the values of YBX-1, IL-6 and CA 15-3 in metastatic BC, non-metastatic BC and control groups. The median serum YBX-1 level was 11.15 ± 3.86 pg/ml in metastatic BC patients and 7.53 ± 1.12 pg/ml in

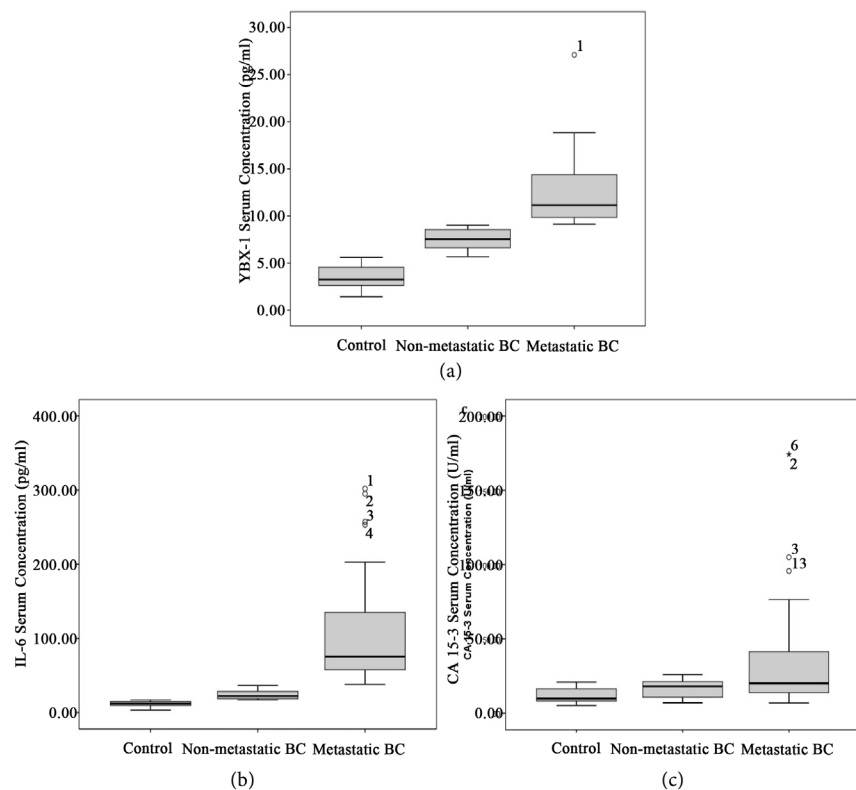


Figure 1. Quantitative levels of (a) YBX-1; (b) IL-6 and (c) CA 15-3 in metastatic BC, non-metastatic BC and control groups.

Table 1. Clinicopathological parameters of breast cancer patients.

| Clinicopathological Characteristics | BC Patients | |
|---|--------------|-------|
| | No. | % |
| Age | 52.23 ± 9.53 | |
| Menopausal Status | | |
| Pre-menopausal | 13 | 21.7% |
| Post-menopausal | 47 | 78.3% |
| Family History | | |
| Negative | 51 | 85% |
| Positive | 9 | 15% |
| Type of Surgery | | |
| Modified Radical Mastectomy | 60 | 100% |
| Tumor Type | | |
| Invasive Ductal Carcinoma | 60 | 100% |
| Estrogen Receptor (ERα) Expression | | |
| - | 7 | 11.7% |
| + | 20 | 33.3% |
| ++ | 25 | 41.7% |
| +++ | 8 | 13.3% |
| Progesterone Receptor (PR) Expression | | |
| - | 10 | 16.7% |
| + | 24 | 40% |
| ++ | 20 | 33.3% |
| +++ | 6 | 10% |
| Her2/neu Expression | | |
| - | 35 | 58.4% |
| + | 8 | 13.3% |
| ++ | 8 | 13.3% |
| +++ | 5 | 8.3% |
| Unknown | 4 | 6.7% |
| Tumor size (Cm) | | |
| T1 (≤ 2) | 17 | 28.3% |
| T2 (2 - ≤ 5) | 37 | 61.7% |
| T3 (> 5) | 6 | 10% |
| Lymph Nodes Involvement | | |
| N0 | 14 | 23.3% |
| N1 (1 - 3) | 25 | 41.7% |
| N2 (4 - 9) | 3 | 5% |
| N3 (> 9) | 18 | 30% |

Continued

| | | |
|---------------------------|----|-------|
| Vascular Invasion | | |
| - | 2 | 3.3% |
| + | 58 | 96.7% |
| Histological Grade | | |
| II | 55 | 91.7% |
| III | 5 | 8.3% |
| Clinical Stage | | |
| I | 1 | 1.7% |
| II | 18 | 30% |
| III | 11 | 18.3% |
| IV | 30 | 50% |
| Metastasis | | |
| No metastasis | 30 | 50% |
| Metastasis | 30 | 50% |
| Site of Metastasis | | |
| Bone | 9 | 30% |
| Lung | 8 | 26.7% |
| Liver | 6 | 20% |
| Other sites | 7 | 23.3% |

non-metastatic patients which was significantly higher than its level in control group (3.26 ± 1.33 pg/ml) with p values of <0.001 . The YBX-1 level in metastatic BC patients was also significantly higher than its levels in non-metastatic patients ($p \leq 0.001$). Similar pattern was also observed in IL-6 levels in all studied groups where its median value 75.48 ± 14.22 pg/ml in metastatic group and 22.21 ± 5.70 pg/ml in non-metastatic groups. Again, both groups were significantly elevated compared to the 12.00 ± 3.30 pg/ml level of control group ($p \leq 0.001$). IL-6 was also significantly elevated in metastatic than non-metastatic patients ($p \leq 0.001$). Regarding CA 15-3 serum levels, its median values in metastatic (20.10 ± 44.24 pg/ml) and non-metastatic (18.21 ± 5.88) groups were significantly elevated than control group (16.00 ± 7.39) ($p = 0.017$ and 0.001 respectively), however, it failed to differentiate between both cancer patients' groups ($p = 0.442$).

3.3. Correlations of YBX-1, IL-6 and CA 15-3 with Clinicopathological Characteristics

Stratification analysis revealed that serum levels of YBX-1, IL-6 and CA 15-3 in all breast cancer patients ($n = 60$) with clinicopathological parameters are represented in **Table 2**. CA 15-3 didn't show any significant association or correlation with all clinicopathological parameters. On the other hand, YBX-1 and

Table 2. Stratification analysis of Y-Box1, IL-6, and CA 15.3 in patients with different clinicopathological status.

| | No. | Y-Box1 | IL-6 | CA 15.3 |
|--------------------------------|-----|----------------------|-------------------------|------------------------|
| Menopausal | | | | |
| Pre | 13 | 8.64 (5.75 - 12.97) | 43.10 (17.22 - 99.83) | 21.16 (7.15 - 56.34) |
| Post | 47 | 10.44 (5.68 - 27.1) | 74.01 (16.78 - 302) | 30.05 (0.02 - 174.23) |
| U(p) | | 228.5 (0.167) | 229 (0.170) | 303.5 (0.971) |
| ER | | | | |
| - | 7 | 10.30 (6.22 - 18.84) | 79.7 (18.52 - 295.04) | 47.77 (10.20 - 174.23) |
| + | 20 | 9.64 (5.73 - 27.10) | 63.46 (16.78 - 302) | 18.22 (7.02 - 41.34) |
| ++ | 24 | 10.76 (5.68 - 18.49) | 74.54 (16.78 - 257.22) | 33.36 (7.43 - 174.23) |
| +++ | 8 | 8.82 (6.79 - 11.72) | 40.70 (19.39 - 81.57) | 20.18 (7.15 - 42.69) |
| H(p) | | 3.77 (0.288) | 3.89 (0.273) | 2.14 (0.545) |
| PR | | | | |
| - | 10 | 11.54 (6.22 - 27.10) | 91.17 (18.52 - 302) | 39.04 (10.2 - 174.23) |
| + | 23 | 9.87 (5.75 - 18.49) | 59.22 (17.22 - 257.22) | 23.00 (7.43 - 105.01) |
| ++ | 20 | 9.92 (5.68 - 15.94) | 71.85 (16.78 - 253.3) | 29.16 (7.02 - 174.23) |
| +++ | 6 | 8.78 (5.68 - 27.10) | 38.45 (19.39 - 69.83) | 26.48 (10.75 - 174.23) |
| H(p) | | 0.50 (0.919) | 0.59 (0.899) | 4.87 (0.181) |
| Her2/neu | | | | |
| - | 35 | 10.15 (5.73 - 27.1) | 64.62 (16.78 - 302) | 27.67 (7.02 - 174.23) |
| + | 8 | 10.71 (5.68 - 18.84) | 109.45 (16.78 - 295.04) | 36.55 (8.73 - 174.23) |
| ++ | 8 | 8.59 (6.06 - 12.9) | 29.55 (17.22 - 59.83) | 16.60 (7.15 - 39.03) |
| +++ | 5 | 9.98 (5.68 - 27.10) | 53.74 (22.34 - 102) | 29.22 (8.83 - 76.60) |
| H(p) | | 1.39 (0.708) | 1.52 (0.679) | 3.77 (0.287) |
| Tumor size | | | | |
| T1 (≤2) | 17 | 10.19 (5.73 - 18.84) | 64.28 (17.22 - 257.22) | 21.44 (7.02 - 105.01) |
| T2 (2 - ≤5) | 37 | 9.92 (3.94 - 27.10) | 71.48 (16.78 - 302.0) | 30.64 (7.15 - 174.23) |
| T3 (>5) | 6 | 9.77 (6.76 - 15.94) | 50.19 (18.52 - 88.52) | 32.62 (14.68 - 95.76) |
| H(p) | | 0.12 (0.943) | 0.20 (0.0.905) | 2.44 (0.0.295) |
| Lymph nodes involvement | | | | |
| N0 | 13 | 8.94 (7.37 - 10.80) | 47.02 (20.70 - 128.96) | 20.87 (8.83 - 42.69) |
| N1 (1 - 3) | 24 | 9.06 (5.68 - 12.99) | 44.45 (16.78 - 102) | 22.81 (7.02 - 95.76) |
| N2 (3 - 9) | 3 | 7.32 (6.22 - 8.02) | 21.57 (18.52 - 23.74) | 19.01 (14.68 - 23.28) |
| N3 (>9) | 19 | 12.49 (6.06 - 27.10) | 111.86 (17.22 - 302.0) | 42.03 (7.43 - 174.23) |
| H(p) | | 5.32 (0.150) | 5.30 (0.151) | 0.98 (0.806) |
| Histological Grade | | | | |
| II | 55 | 9.60 (5.68 - 18.84) | 59.87 (16.78 - 295.04) | 27.03 (7.02 - 174.23) |
| III | 2 | 15.01 (8.57 - 27.10) | 149.13 (28.96 - 302) | 40.17 (17.94 - 95.76) |
| H(p) | | 56.0 (0.029)* | 56.1 (0.027)* | 69.0 (0.068) |

Continued

| | | Clinical Stage | | |
|-------------|----|------------------------|-------------------------|-----------------------|
| I | 1# | 8.09# | 26.35# | 16.67# |
| II | 18 | 7.68 (5.68 - 9.02) | 24.68 (16.78 - 35.04) | 17.75 (8.73 - 26.05) |
| III | 11 | 7.17 (6.06 - 9.02) | 21.53 (17.22 - 36.35) | 16.38 (7.15 - 23.28) |
| IV | 30 | 12.58 (9.14 - 27.10) | 111.04 (38.09 - 302) | 39.04 (7.02 - 174.23) |
| H(p) | | 60 (<0.001)* | 60 (<0.001)* | 1.22 (0.747) |
| | | Vascular Invasion | | |
| Negative | 2 | 9.35 (9.22 - 9.49) | 45.26 (42.0 - 48.52) | 23.39 (17.65 - 29.13) |
| Positive | 28 | 10.07 (5.68 - 27.10) | 68.07 (16.78 - 302) | 28.29 (7.02 - 174.23) |
| U(p) | | 52.0 (0.824) | 52.0 (0.824) | 42.0 (0.547) |
| | | Site of Metastasis | | |
| Bone | 9 | 14.64 (9.02 - 18.49) | 146.40 (36.35 - 257.22) | 48.02 (7.43 - 105.01) |
| Lung | 8 | 12.62 (9.49 - 15.94) | 117.92 (48.52 - 253.30) | 37.06 (7.02 - 56.34) |
| Liver | 6 | 12.29 (9.23 - 15.94) | 108.30 (46.35 - 253.30) | 43.98 (14.13 - 95.76) |
| Other sites | 7 | 10.17 (9.22 - 81.57) | 60.07 (42 - 81.57) | 25.55 (7.02 - 81.56) |
| H(p) | | 7.91 (0.048)* | 7.05 (0.070) | 4.70 (0.195) |

U: Mann Whitney test, H: H for Kruskal Wallis test. *: Statistically significant at $p \leq 0.05$, #: Excluded from the relation due to small number of case ($n = 1$).

IL-6 showed a significant association histological grade ($p = 0.029$, 0.027 respectively) and clinical stage ($p < 0.001$) (Figure 2). Spearman correlations also revealed a significant positive correlation between both markers with patients' histological grade ($r_s = 0.282$, $p = 0.029$, $r_s = 0.284$, $p = 0.028$) and clinical stage ($r_s = 0.789$, $p < 0.001$, $r_s = 0.788$, $p < 0.001$) for YBX-1 and IL-6 respectively.

Further investigation of the levels of YBX-1 and IL-6 in metastatic group ($n = 30$) showed that they are further associated with lymph nodes involvement ($p = 0.001$, 0.025) and vascular invasion ($p = 0.046$, 0.003) as presented in Figure 3. Metastasis site-specific analysis showed a significant difference between YBX-1 level ($H = 7.91$, $p = 0.048$), however, none of the major metastasis sites (Bone, lung and liver) showed a significant elevation than the other. Furthermore, no particular site of metastasis showed a significant elevation in IL-6 and CA 15-3 than the others ($H = 7.05$, $p = 0.070$ and $H = 4.70$, $p = 0.195$ respectively).

3.4. Correlation of YBX-1 and IL-6 Serum Concentrations in BC Patients

In the overall breast cancer patients ($n = 60$) the median YBX-1 serum levels was 9.08 ± 0.50 pg/ml and IL-6 was 37.22 ± 9.08 . IL-6 levels were elevated in patients with higher YBX-1 level. As shown in Figure 4, Spearman correlation between YBX-1 and IL-6 levels revealed that there was a significant positive correlation between the levels of both markers ($r_s = 0.615$, $p < 0.001$).

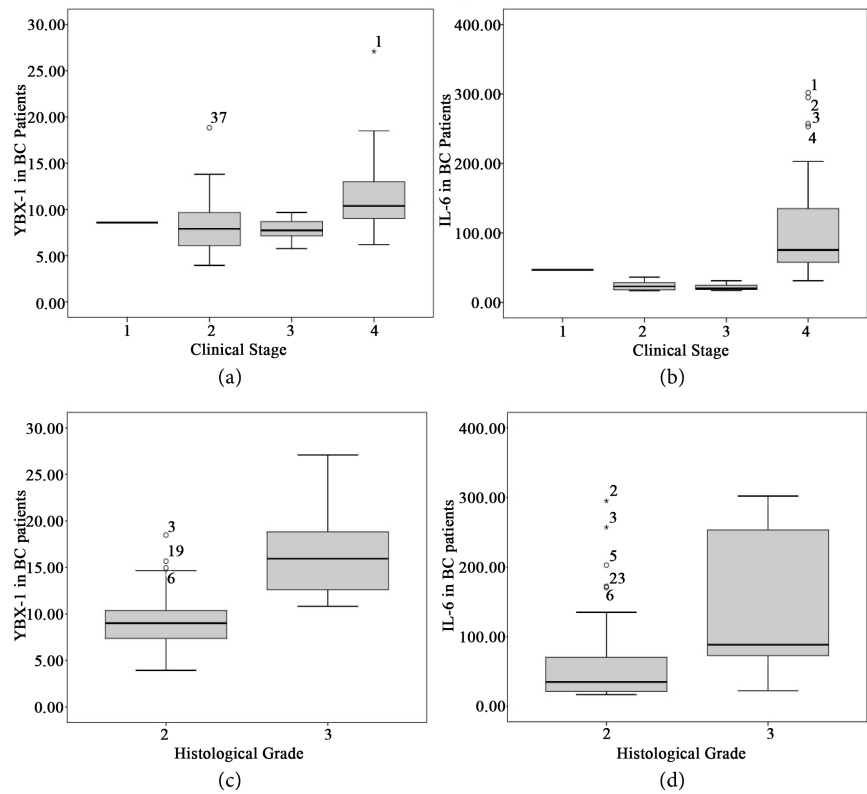


Figure 2. Correlations between Y-Box1 and IL-6 Levels with clinical stage ((a), (b)) and histological grade ((c), (d)) in BC patients.

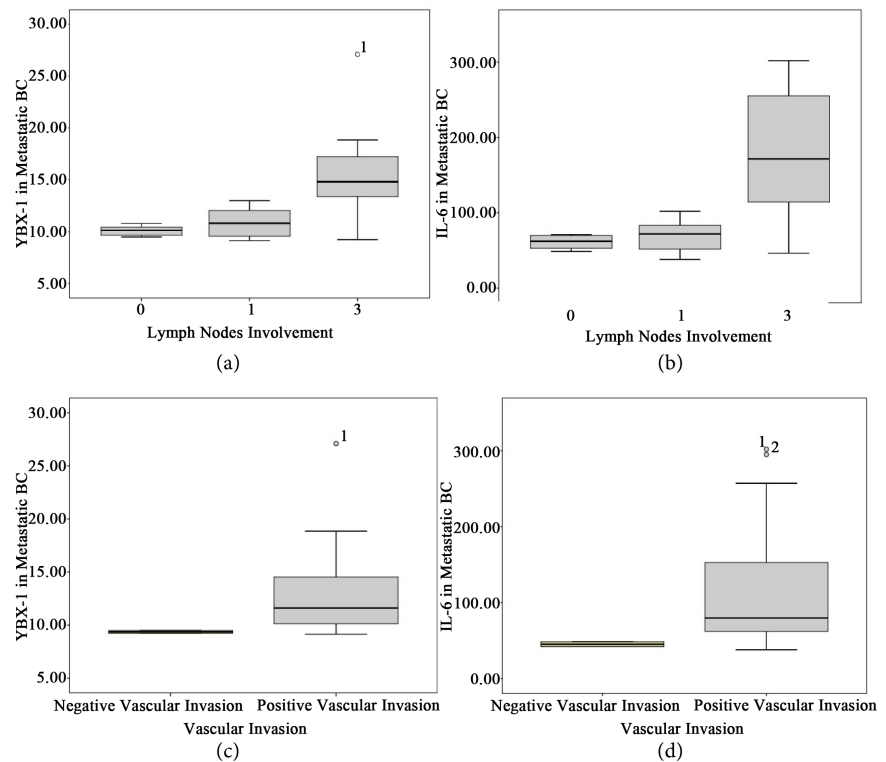


Figure 3. Association between Y-Box1 and IL-6 Levels with lymph nodes involvement ((a), (b)) and vascular invasion ((c), (d)) in metastatic BC patients.

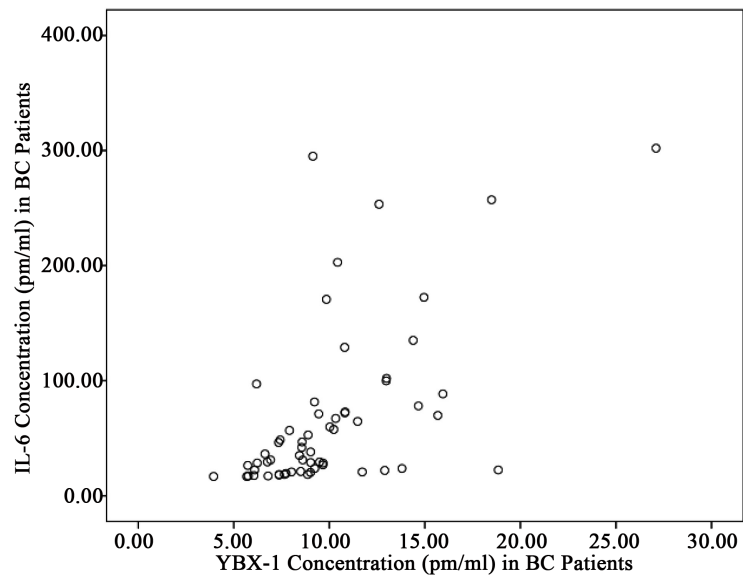


Figure 4. Correlation between Y-Box1 and IL-6 Levels in BC patients.

3.5. YBX-1 and IL-6 Have Superior Sensitivity and Specificity over CA 15-3 in Predicting BC Metastasis

Investigating the sensitivity and specificity of YBX-1 and IL-6 in predicting BC metastasis we constructed a ROC curve against CA15-3. The ROC curve for YBX-1 (**Figure 5(a)**) had an area below the curve of 0.892 ($p < 0.001$) with confidence interval (0.0826 - 0.958). The cut-off point, or Youden Index, was 8.5 pg/ml. The model has high specificity (79.3%) and sensitivity (86.7%). Regarding the IL-6, area below the curve was 0.996 ($p = 0.004$) with confidence intervals (0.988 - 1.000). The Youden index was 8.74 pg/ml with 100% sensitivity and 90.9% specificity (**Figure 5(b)**).

4. Discussion

Regardless of the current advances in diagnosis and treatment, recurrence and metastasis still threaten a high number of breast cancer patients and contribute to the high disease related mortality rates. In the present study, we primarily aimed to quantify serum levels of YBX-1 and IL-6 in breast cancer patients compared to CA 15-3 which is routinely used to follow up BC patients. Our results showed that both YBX-1 and IL-6 are significantly elevated in BC patients and their levels are even more elevated with cancer metastasis. On the other hand, although CA 15.3 was significantly elevated in both cancer groups, its levels in metastatic and non-metastatic patients were not significantly different.

The elevated levels of YBX-1 especially in metastatic BC patients is supported by the strong evidence on its role in breast cancer progression and aggressiveness. A meta-analysis showed that the patients with increasing YBX-1 expression are at high risk of relapse and metastasis compared with those with low YBX-1 expression [22]. Although most previous data focused on evaluating the nuclear and/or cytoplasmic expression of YBX-1 in tumor cells, very scarce studies have

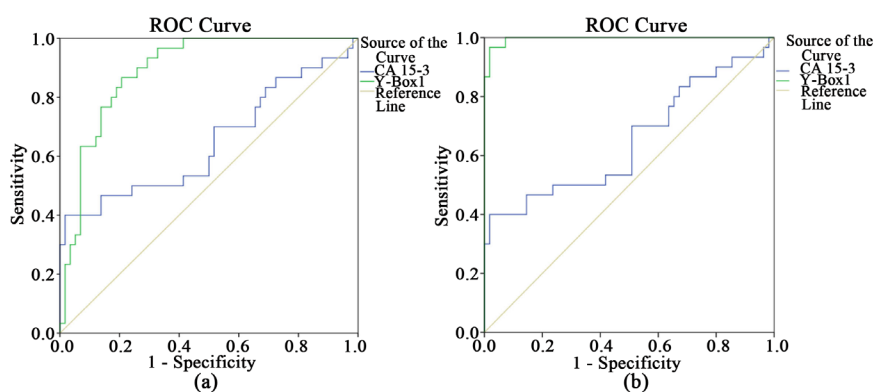


Figure 5. ROC curves for Y-Box1 and IL-6 levels against CA15-3 in metastatic BC patients.

focused on evaluating its serum levels. YBX-1 is secreted through an non-classical vesicle-mediated pathway, from mesangial and monocytic cells under lipopolysaccharide-induced inflammatory stress [23]. In 2014, Tacke *et al.* have reported that YBX-1/p-18 fragment is more prevalent in the plasma of patients with different malignancies including lung, breast and hematological malignancies [24]. Several studies have also supported the role of IL-6 in BC however their results were contradicting. Our results suggest that IL-6 is associated with poor clinical outcome. These results are supported with the results of Ma *et al.* (2017) who reported the association of elevated serum IL-6 with poor prognosis [25]. Nevertheless, other reports have characterized IL-6 as a marker of good prognosis [26]. This apparent contradiction might be attributed to the difference in patients' stratification according to hormonal status. Studies conducted on patients with mixed ER/PR status showed similar results to ours unlike studies that focused on ER+ patients alone [27]. It is worth noting that ER+ BC cells have been reported to produce minimal autocrine IL-6 and are dependent on paracrine IL-6 from within the tumor microenvironment including that produced by bone fibroblasts and adipose tissue [9] [28]. IL-6 exerts its effects on ER+ BC cells via the phosphorylation of tyrosine 705 of signal transducer and activator of transcription (STAT) 3 and consequently, promotes growth and invasion [29].

As for the relation between YBX-1 and IL-6 our results indicated a significant positive correlation between the two parameters. These results are supported by previous reports suggesting that IL-6 is a transcriptional target for YBX-1 activity [30]. The results of Castellana *et al.* supported that forced expression of YBX-1 increased IL-6 levels in three BC cell lines by directly binding and stabilizing the short-lived mRNA of IL-6 [31]. The interplay between YBX-1 and IL-6 seems to create a positive feed-forward loop leading to the activation of EMT-like features of cancer cells, including increased motility thus promoting cell invasion and metastasis [32].

The significant elevation of YBX-1 and IL-6 in breast cancer patients was further analyzed by stratifying the patients according to their clinicopathological

characteristics. Our results indicated that YBX-1 and IL-6 serum levels were significantly correlated to advanced histological grade and poor clinical stage in BC patients. Further analysis indicated that both parameters were significantly associated with increased lymph nodes involvement and positive vascular invasion in metastatic breast cancer patients which supports our hypothesis of their central role in cancer invasion and metastasis. In agreement with our results, Nuclear YB-1 expression was found to be correlated with disease stage, tumor diameter, stromal invasion, and lymph-node metastasis in cervical cancer. It was also found to be correlated with epithelial growth factor receptor expression which implies to its role in processes associated with tumor development and progression, such as cell growth, inhibition of apoptosis, cell migration, and angiogenesis [18]. YBX-1 expression level was also correlated with tumor grade and invasiveness in bladder carcinoma [33]. YBX-1 has also been reported to be associated with “stem cell-like” tumor phenotype based on retrospective immunohistochemical study of paraffin-embedded breast cancer tissues [34]. A recent study reported a co-elevation of serum YBX-1 and IL-6 in breast cancer patients that was significantly correlated to bone metastasis [35].

Regarding the utility of YBX-1 and IL-6 as clinical biomarkers, we analyzed their sensitivity and specificity in identifying metastatic breast cancer patients compared to CA 15-3 which is routinely used as a follow up and prognostic marker. Our results indicated that YBX-1 and IL-6 had significantly higher sensitivity and specificity than CA 15-3 which implies their potential role as prognostic markers for breast cancer. The prognostic role of YBX-1 and IL-6 has been referred to in a number of previous studies on various types of cancers including ovarian [36], prostate [37], acute lymphoblastic leukemia [38] and multiple myeloma [39]. However, most of these studies focused on analysis of either fresh or paraffin-embedded tissues with few exceptions which focused on minimally invasive samples like serum or plasma [35] [40]. Our results therefore support the evidence that serum YBX-1 and IL-6 levels can be used as valuable biomarkers of breast cancer with a strong potential of predicting metastasis.

Our study experienced some limitations including the small sample size, which might have caused less statistical power. Another limitation is that we didn't include disease-free and overall survival follow-up and analysis to allow a more detailed investigation of their prognostic significance. Therefore, further study with a larger sample size and patients' follow-up is needed.

5. Conclusion

From the results of this study, we can conclude that serum YBX-1 and IL-6 are potential biomarkers of breast cancer patients with significant correlation with poor clinicopathological characteristics. Serum YBX-1 and IL-6 have superior sensitivity and specificity compared to CA15-3 and can serve as a potential follow up and prognostic marker.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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