The biomarker significance of three chemokines (CXCL8, CXCL10, and CXCL16) was evaluated in sera of 45 breast cancer (BC) and 28 benign breast lesion (BBL) patients, as well as 20 control women. Clinical stage and tumor expression of estrogen (ER), progesterone (PR), and human epidural growth factor receptor-2 (HER-2) receptors were considered in this evaluation. The results demonstrated that CXCL8, CXCL10, and CXCL16 showed a significant increased median in BC and BBL patients compared to control (CXCL8: 47.3 and 25.7 vs. 15.0; CXCL10: 37.6 and 30.7 vs. 13.1; CXCL16: 27.9 and 25.2 vs. 19.2 pg/mL, respectively). The increased levels of CXCL8 and CXCL16 were more pronounced in triple-negative patients, respectively. Binary logistic regression analysis revealed that CXCL8 was a significant predictor of BC, and such prediction was more depicted in triple-negative patients. The receiver operating characteristic analysis also revealed that CXCL8 recorded an area under curve of 0.928 in BC patients. In conclusion, CXCL8 is a potential biomarker for BC, especially when ER, PR, and HER-2 expression is considered. In this context, the predictive significance of CXCL8 in influencing BC progression is suggested in triple-negative patients.

Keywords: Benign breast lesion, Breast cancer, CXCL10, CXCL16, Date.
Introduction

Breast cancer (BC) is the most common cancer in women and it is a late stage disease that shows a high mortality and morbidity rate [1]. In 2012, 1,671,149 new cases of BC were diagnosed, with 521,907 death-related cases [2]. In Iraq, BC is the commonest type of female cancers and in 2012, 4,115 cases were reported, accounting for approximately 30% of the registered cancers in women [3].

There are different BC-associated risk factors, for instance, gender, estrogen, family history, aging, multiple gene mutations and lifestyle, which can increase the risk of developing such female malignancy [4]. However, host immunity can also increase (protective) or decrease (suggering) BC growth, thus, immune-editing pathways are a dynamic process, in which tumor cells and the host immune response are involved, and marked by inflammatory reactions. Inflammation, therefore, plays a critical role in tumor onset, development, angiogenesis, and cell migration, and cytokines have been suggested to exert an important role in each event of BC progression [5].

Chemokines are members of a superfamily of chemotactic cytokines initially characterized because of their association with inflammatory responses via stimulation of leukocyte migration and adhesion during inflammation. They also influence other cellular functions especially those that are related to tumor progression; for instance, proliferation, angiogenesis, malignant transformation and cancer metastasis [6]. In BC, chemokines have been reported to be associated with the enhancement of a pro-inflammatory microenvironment and can direct metastasis in the breast. They are also involved in BC progression, favoring growth and proliferation of local tumor cells. Several chemokines and chemokine receptors have been suggested to participate in these processes, and CXCL8, CXCL10 and CXCL16 are three important chemokines that may have a role in the establishment of malignancy in female breast [7].

Materials and Methods:

Patients and control

The study was approved by the Ethics Committee at the Iraqi Ministry of Health. It involved 63 women with breast tumor, and according to the type of tumor, they were distributed as BC and benign breast lesion (BBL) groups. The first included 45 patients, while the second composed of 18 patients. The age range of BC patients was 25 - 78 years (mean ± standard deviation: 50.6 ± 12.2 years), while such range in BBL patients was 16 - 52 years (33.1 ± 9.7 years). The patients were referred to the Oncology Teaching Hospital in Baghdad during March 2016 - March 2017. The diagnosis was made by the consultant medical staff, which was based on physical breast examination, ultrasonography, with or without mammography and fine needle aspiration cytology (Triple Assessment Technique). Based on the histopathological examination, all BC tumors were grouped under invasive ductal carcinoma (IDC), while BBLs were fibroadenomas. The TNM (Tumor-Node-Metastases) system was employed to determine the clinical stage of BC. It was based on three major morphological features: tumor size (T), regional lymph node involvement (N) and distant metastases (M). Based on such system, three clinical stages (I, II and III) were identified, but for the purpose of statistical analysis, stages I and II were considered as one group. The BC tumors were also characterized in terms of their immunohistochemical expression of ER, PgR, and HER-2 receptors [Table 1]. In addition to patients, 20 apparently healthy married women were included in the study (control group), and their age range was 20 - 45 years (32.1 ± 7.1 years).

<table>
<thead>
<tr>
<th>Tumor size</th>
<th>N (%)</th>
<th>ER expression</th>
<th>Data</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1, T2</td>
<td>56 (86.4)</td>
<td>Positive</td>
<td>52 (81.1)</td>
<td></td>
</tr>
<tr>
<td>T1, T4</td>
<td>9 (13.6)</td>
<td>Negative</td>
<td>12 (19.8)</td>
<td></td>
</tr>
<tr>
<td>Regional lymph node involvement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>33 (53.3)</td>
<td>Positive</td>
<td>30 (48.3)</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>12 (18.7)</td>
<td>Negative</td>
<td>12 (18.3)</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>33 (53.3)</td>
<td>Positive</td>
<td>22 (35.4)</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>12 (18.7)</td>
<td>Negative</td>
<td>12 (18.3)</td>
<td></td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II</td>
<td>21 (60.0)</td>
<td>Tripe-negative expression</td>
<td>4 (6.9)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>14 (40.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

23: Number: ER, Estrogen; PgR, Progesterone; HER-2: Human epidermal growth factor receptor-2.

References should be mentioned in order from 1 to the last number mentioned. References are numbered and cited as they appear in the text using superscript e.

In the case of combining two references, write as follows: 1, 2

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Tables and shapes are placed in the results in the sequence mentioned, not at the end of the manuscript

The titles of the tables are written at the top of the table, pt 11 and type Bold, taking into consecration writing the name of the table and its number appended with a dot, as shown.

The tables should be in (style 1) format within the shapes of tables in MS word software, as shown.

The text of the table’s contents must be 10 in size.
BBL (OR = 1.23; 95% CI: 1.03 - 1.46; p-value = 0.024). CXCL8 prediction was more pronounced in BC patients with a negative expression of ER and PgR, especially triple-negative patients (OR = 2.51; 95% CI: 1.02 - 6.19; p-value = 0.046). BBL had a further predictor that was CXCL10 (OR = 1.14; 95% CI: 1.03 - 1.2; p-value = 0.012). The latter chemokine was also associated with a significant OR in clinical stage III patients (OR = 1.19; 95% CI: 1.02 - 1.37; p-value = 0.025). CXCL16 was only observed to have a significant OR in HR-2+ve patients (OR = 1.13; 95% CI: 1.03 - 1.23; p-value = 0.007) in Eq.1 as ascil(s)/iteration ….

Table 4. Binary logistic regression analysis of CXCL8, CXCL10 and CXCL16 serum levels as predictor variables in breast cancer and benign breast lesion patients as outcome variables.

<table>
<thead>
<tr>
<th>Category*</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total BC</td>
<td>1.23 (1.03 - 1.46)</td>
<td>0.011</td>
<td>1.12 (0.97 - 1.28)</td>
<td>0.113</td>
</tr>
<tr>
<td>Clinical stage III</td>
<td>0.96 (0.84 - 1.10)</td>
<td>0.541</td>
<td>1.19 (1.02 - 1.37)</td>
<td>0.026</td>
</tr>
<tr>
<td>ER-ve</td>
<td>1.25 (1.03 - 1.46)</td>
<td>0.021</td>
<td>0.90 (0.80 - 1.03)</td>
<td>0.116</td>
</tr>
<tr>
<td>PgR-ve</td>
<td>1.16 (1.01 - 1.34)</td>
<td>0.039</td>
<td>0.98 (0.88 - 1.09)</td>
<td>0.735</td>
</tr>
<tr>
<td>HER-2+ve</td>
<td>0.94 (0.82 - 1.06)</td>
<td>0.201</td>
<td>1.02 (0.91 - 1.14)</td>
<td>0.737</td>
</tr>
<tr>
<td>Triple-negative</td>
<td>2.31 (1.02 - 5.19)</td>
<td>0.046</td>
<td>1.08 (0.77 - 1.51)</td>
<td>0.654</td>
</tr>
<tr>
<td>BBL</td>
<td>1.23 (1.03 - 1.46)</td>
<td>0.024</td>
<td>1.14 (1.03 - 1.2)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

BC: Breast cancer; ER: Estrogen; PgR: Progesterone; HER-2: Human epidermal growth factor receptor-2; -ve: Negative; BBL: Breast lesion lesion; OR: Odds ratio; CI: Confidence interval; p: Probability. *Control was the reference category for total BC and BBL, while for BC subgroups; the corresponding subgroup was the reference category.

When the ROC analysis was carried out, an AUC value greater than 0.9 was recorded for CXCL8 and CXCL10 in BC patients (0.998 and 0.992, respectively), while among BBL patients, it was for CXCL8 only (0.902). The highest sensitivity and specificity were obtained for CXCL8 in BC patients (95.6 and 95.0%, respectively) in Fig. 1.

Figure 1. Receiver operating characteristic curve (area under curve; AUC) of CXCL8, CXCL10 and CXCL16 serum levels among breast cancer and benign breast lesion patients versus control.

Discussion:
The presented results suggest the predictive significance of CXCL8 and CXCL10 as biomarkers for BC and BBL, being more evident in malignant lesions. Earlier studies indicated relevant findings and these chemokines have been reported to be associated with a progression of the female breast malignancy. However, two observations are of note in present study: significant increased levels of CXCL8 in triple-negative and CXCL16 in HER-2+ve BC patients.

With respect to cancer, two opposite functional roles of CXCL8 have been suggested: it enhances the immunoregulatory potentials against carcinogenesis, and can also modify the tumour microenvironment to enhance tumourigenesis. The latter role is more dominant in BC compared to the former because BC cells have been found to express two receptors (CXCR1 and CXCR2) for CXCL8. The interaction between CXCL8 and its cognate receptors has been associated with initiation and development of BC. In addition, it has been reported that an increased level of CXCL8 is mostly detected in patients exhibiting the ER-ve, PgR-ve and HER-2+ve phenotypes. An observation that is strongly enhanced by the present study results, whereby patients displaying the triple-negative subtype demonstrated a significant increased serum level of CXCL8 compared to patient’s positive for one or more of these receptors. In this context, it has been found that a down-regulation of CXCL8 was associated with a significant reduction in the density of microvessels in ER-ve BC patients.

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*Equations should be written using MS word software, not pasted as image

The equation number must be written without parentheses

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* MS Excel charts should be added as clickable charts, and not copy pasted as image.

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Figure appended with a figure number and a dot
progression of BC is suggested, especially in triple-negative patients. However, the results of present study were limited by the sample size of patients and control.

Acknowledgment:
The cooperation of the medical staff at the Oncology Teaching Hospital in Baghdad is appreciated.

Authors' declaration:
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine own. Besides, the Figures and images, which are not mine own, have been given the permission for re-publication attached with the manuscript.
- The author has signed an animal welfare statement.
- Authors sign on ethical consideration’s approval
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors’ contributions statement:
This work was carried out in collaboration between all authors. A.A diagnosis the cases then collected the samples and doing the tests. B.B wrote and edited the manuscript with revisions idea. N.A and B.B analysis the data with revisions idea. All authors read and approved the final manuscript.

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