Title of MS

AAAAAAAAAAAA [1] *MMMMMMMMMMM* [2] *NNNNNNNNN A* [3] *BBBBBBB* [4*]

[1] Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq.
[2] College of Medicine, University of Baghdad, Baghdad, Iraq.
[3] National Cancer Research Center, University of Baghdad, Baghdad, Iraq.
[4] Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq
*Corresponding author: d.... @sc.uobaghdad.edu.iq
E-mails address: s.... @sc.uobaghdad.edu.iq , t.... @sc.uobaghdad.edu.iq.
*ORCID ID: https://orcid.org/0000-0002-2445-2242

Abstract:

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 (PgR) and human epidermal 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 and HER-2 positive 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 0.998 in BC patients. In conclusion, CXCL8 is a potential biomarker for BC, especially when ER, PgR 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.

عنوان البحث

1)))))))) [1] ممممممم [2] ممممممم [3] عععععععه [4]

[1] قسم التقنيات الاحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق.
[2] كلية الطب، جامعة بغداد، بغداد، العراق.
[3] المركز الوطني لبحوث السرطان، جامعة بغداد، بغداد، العراق.
[4] وحدة البحوث البيولوجية للمناطق الحارة، كلية العلوم، جامعة بغداد، بغداد، العراق.

الخلاصة:

قيمت الاهمية الواسم-حياتية لثلاث من الحركيات الكيميائية (CXCL8 وCXCL10 وCXCL16) في مصول 45 من مريضات سرطان الثدي و28 من مريضات ورم الثدي الحميد و20 من نساء السيطرة. واعتمدت المرحلة السريرية وتعبير الورم لمستقبلات Estrogen وProgesterone وعامل النمو الشري HER-2 في هذا التقييم. اظهرت النتائج زيادة معنوية في متوسط CXCL8 وCXCL10 وCXCL16 لمريضات سرطان الثدي ومريضات ورم الثدي الحميد مقارنة بالسيطرة (CXCL8: 47.3 و25.7 مقابل 15.0؛ CXCL10: 37.6 و30.7 مقابل 13.1؛ CXCL16: 27.9 و25.2 مقابل 19.2 بيكوغرام/مل، على التوالي). وكانت زيادة مستوى CXCL8 وCXCL16 اكثر وضوحا في المريضات ثلاثية-سالبة التعبير والموجبة للمستقبل HER-2، على التوالي. اظهر تحليل الانحدار اللوجستي التنائي اهمية CXCL8 كعامل تنبؤي لسرطان الثدي وان ذلك اكثر وصفا في المريضات ثلاثية سالبة التعبير. وفضلا عن ذلك فقد اظهر تحليل خصائص المستقبل التشغيلية باحتلال CXCL8 مساحة تحت المنحنى قدرها 0.988 في مريضات سرطان الثدي. يمكن الاستنتاج بان CXCL8 ذو اهمية لواسم - حياتي لسرطان الثدي خصوصا عند الاخذ بنظر الاعتبار تعبير الورم لمستقبلات Estrogen وProgesterone وعامل النمو الشري HER-2. وفي هذا الصدد، فان الدراسة تقترح الاهمية التنبؤية للحركي الكيميائي-8 في تطور سرطان الثدي لدى المريضات ثلاثية سالبة التعبير.

الكلمات المفتاحية: سرطان الثدي، ورم الثدي الحميد، الحركيات الكيميائية 8 و10 و16.

1. **Paper size: A4, the margins should be 0.79" in all directions, made with MS word 2010 or later**
2. **Title: pt 14, bold, centered**
3. **Author's names should be 12 in size, Bold and Italic**
4. **Author's Affiliation, E-mails and ORCID ID's must be 10 in size and written in order, as shown.**
5. **The manuscript' main titles (abstract, introduction, material, methods of work, results, and discussion): pt 12, bold and non-numbered**
6. ***Article text should be 11 in size**
   ***Abstract words should be 250 to 300 words.**
7. **The keywords are written under the Abstract (5 keywords at least) in order sequence using the English alphabet.**
8. **Arabic title should be translated professionally with a size of 14, type Bold.**
9. **Author's names in Arabic should be 12 in size and type Bold**
10. **Author's addresses in Arabic should be 11 in size**
11. **The Arabic abstract is written in size 12 for the title, type Bold, and size 11 for the content, and it is translated in a professional way, not translated using google translator.**
12. **The Arabic keywords are written in the same order as mentioned in the English language, and must be 11 in size with Bold title.**

Introduction:

Breast cancer (BC) is the most common cancer in women and it is a late-stage disease that shows a high morbidity and mortality rates. In 2012, 1,671,149 new cases of BC were diagnosed worldwide, with 521,907 death-related cases [1]. 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 [2].

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[3].

However, host immunity can also increase (promotive) or decrease (suppressive) BC growth, and such 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[4].

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[5]. In BC, chemokines have been reported to be associated with the enhancement of a pro-tumoral microenvironment and can direct metastasis in the breast. They are also involved in BC progression; favoring growth and proliferation of local tumor cells [6]. Several chemokines and chemokine receptors have been suggested to participate in these processes[7], and CXCL8, CXCL10 and CXCL16 are three important chemokines that may have a role in the establishment of malignancy in female breast.

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 28 patients. The age range of BC patients was 25 - 76 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 fibroadenoma. The TNM (Tumor-Node-Metastasis) 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 1. Clinical and laboratory data of patients diagnosed with breast cancer.

Data	N (%)	Data	N (%)
Tumor size		***ER expression***	
T1, T2	38 (86.4)	Positive	32 (71.1)
T3, T4	6 (13.6)	Negative	13 (28.9)
Regional lymph node involvement		***PgR expression***	
Present	33 (73.3)	Positive	30 (66.7)
Absent	12 (26.7)	Negative	15 (33.3)
Distant metastasis		***HER-2 expression***	
Present	33 (73.3)	Positive	22 (48.9)
Absent	12 (26.7)	Negative	23 (51.1)
Clinical stage		***Triple-negative expression***	4 (8.9)
I, II	21 (60.0)		
III	14 (40.0)		

N: Number; ER: Estrogen; PgR: Progesterone; HER-2: Human epidermal growth factor receptor-2

13 All the main headings within the manuscript should be 12 in size, type Bold and non-numbered.

14
* 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^x.
* In the case of combining two references, write as follows: [1, 2]
* In the case of writing a content for more than two references, provided that it does not exceed 4 references combined together, write as follows: [1-4]

Subtitles: pt 11, bold, non-numbered. 15

16
*Tables are mentioned in the manuscript as shown.
*Tables and shapes are placed in the results in the sequence mentioned, not at the end of the manuscript

17 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.

18
*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 = 0.026). CXCL16 was only observed to have a significant OR in HER-2+ve patients (OR = 1.13; 95% CI: 1.03 - 1.23; *p*-value = 0.007) in Eq.1 a= ascii(x)/iteration 1

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.

Category*	CXCL8		CXCL10		CXCL16	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Total BC	1.21 (1.05 - 1.40)	0.011	1.12 (0.97 - 1.28)	0.113	1.09 (0.86 - 1.38)	0.480
Clinical stage III	0.96 (0.84 - 1.10)	0.541	1.19 (1.02 - 1.37)	0.026	0.94 (0.86 - 1.04)	0.220
ER-ve	1.23 (1.03 - 1.46)	0.021	0.90 (0.80 - 1.03)	0.116	0.99 (1.90 - 1.08)	0.737
PgR-ve	1.16 (1.01 - 1.34)	0.039	0.98 (0.88 -1 .09)	0.735	0.98 (0.90 - 1.06)	0.588
HER-2+ve	0.94 (0.82 - 1.06)	0.295	1.02 (0.91 - 1.14)	0.757	1.13 (1.03 - 1.23)	0.007
Triple-negative	2.51 (1.02 - 6.19)	0.046	1.08 (0.77 - 1.51)	0.654	0.88 (0.65 - 1.18)	0.389
BBL	1.23 (1.03 - 1.46)	0.024	1.14 (1.03 - 1.2)	0.012	1.13 (0.95 - 1.34)	0.156

BC: Breast cancer; ER: Estrogen; PgR: Progesterone; HER-2: Human epidermal growth factor receptor-2; -ve: Negative; BBL: Benign breast 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 0.923, respectively), while among BBL patients, it was for CXCL8 only (0.902). The highest sensitivity and specificity were estimated for CXCL8 in BC patients (95.6 and 95.0%, respectively) Fig. 1.

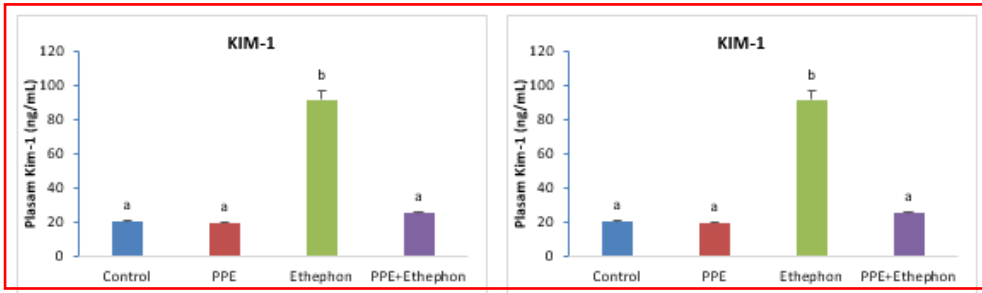


Figure 1. Receiver operating characteristic curve (area under curve; AUC) of CXCL8, CXCL10 and CXCL16 serum levels among breast cancer and benign breast lesion patients *versus* control.

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 tumor 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 micro-vessels in ER-ve BC patients.

***Equations should be mentioned as follows:**
Eq followed by a dot and the number of equation
***Equations should be written using MS word software, not pasted as image**

19

The equation number must be written without parentheses

20

Names of figures and shapes mentioned in the manuscript should be written as follows: Fig appended with a dot and the figure number

21

22

*** Shapes and Figures must be clear.**

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

23

The title of the figure is mentioned below the figure itself, and should be 11 in size, type Bold. As for the name of the figure, it is mentioned as follows:

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 ours. Besides, the Figures and images, which are not mine ours, 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.

References:

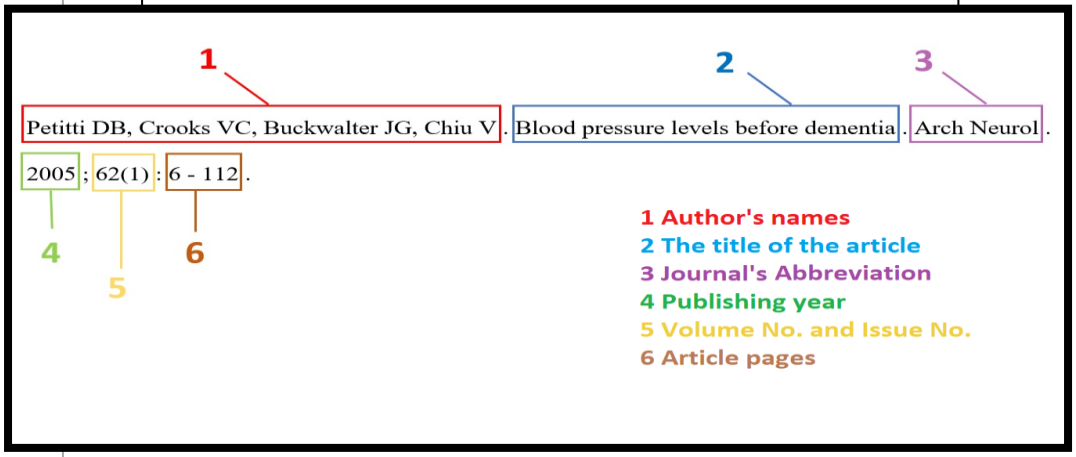
1. Mofijur M, Rasul M G, Hassan N M , Nabi M N. Recent development in the production of third generation biodiesel from microalgae. Energy Procedia 2019; 156: 53-58.
2. Shomal R, Hisham H, Mlhem A, Hassan R, Al-Zuhair S. Simultaneous extraction–reaction process for biodiesel production from microalgae. Energy Rep. 2019; 5: 37-40.
3. Singh D, Sharma D, Soni SL, Sharma S, Sharma PK, Jhalani A. A review on feedstocks, production processes, and yield for different generations of biodiesel. Fuel. 2020 15;262:116553.
4. Anwar F, Rashid U, Ashraf M, Nadeem M. Okra (*Hibiscus esculentus*) seed oil for biodiesel production. Appl. Energy 2010; 87: 779-785.
5. Chang A, Pan JH, Lai NC, Tsai MC, Mochizuki T, Toba M, et al. Efficient simultaneous esterification/transesterification of non-edible Jatropha oil for biodiesel fuel production by template-free synthesized nanoporous titanosilicates. Catalysis Today. 2019 12.
6. Yin Z, Zhu L, Li S, Hu T, Chu R, Mo F, et al. A comprehensive review on cultivation and harvesting of microalgae for biodiesel production: environmental pollution control and future directions. Bioresour. Technol. 2020; 122804.
7. Chozhavendhan S, Singh MV, Fransila B, Kumar RP, Devi GK. A review on influencing parameters of biodiesel production and purification processes. Curr. Opin. Green Sustain. 2020; 1(1):1-6.

Acknowledgment is optional and can be mentioned by the author's choice. 24

25 **The authors' declaration is mentioned as indicated in the points shown exclusively, only changing the location of the approval of the local ethical committee at the Ethical Clearance section, to the place from which the approval was obtained.**

26 *** The contribution of authors must be mentioned as indicated and according to the work of each researcher within the manuscript. For more information on how to write an author contribution, please see the file here**
*** It is not required to write the authors' contribution if there is only one author.**

27 ***References are written only in the English language with Vancouver Citing & Referencing.**
***References font size must be 10.**
***70% of references are recent and are within the last 5 years.**
***Conference references should not exceed 20% of the total references number.**
*** Find articles published in our journal, related to the topic of your research and cite at least two of them.**
*** For abbreviation journals: please use the following as a guide: https://www.ncbi.nlm.nih.gov/nlmcatalog?Db=journals&Cmd=DetailsSearch&Term=currentlyindexed%5BAll%5D**
***Add a DOI link to the articles mentioned in references, at the end after the pages numbers.**
***Please, see the following image for more information on how to write a reference:**



Important notice:

Authors are kindly requested to abide by the above-mentioned instructions. Otherwise, the manuscript will be declined and will not be received a second time, unless all required modifications are completed.