ISSN: 0067-2904

Diagnostic Potential Role of CXCL3 and Leptin Levels in Breast Cancer

Abbas S. Neamah , Fadhel Mohammed Lafta
Department of Biology, College of Sciences, University of Baghdad, Baghdad- Iraq

Received: 2/2/2022 Accepted: 28/4/2023 Published: 30/4/2024

Abstract

The risk of breast cancer development is believed to be attributed to the alterations of a number of key biological components. Within this context, elevated levels of some chemokines that act as growth factors and can promote cancer development. The current study was designed to evaluate CXCL3 (a chemokine C-X-C Motif Ligand 3) and leptin (a peptide hormone synthesized by adipose tissue with cytokine activity) serum of Iraqi breast cancer patients in comparison to healthy controls. A total of 90 participants consisted of 60 patients diagnosed with breast cancer and 30 healthy women as control group were enrolled into this case-control study. Venous blood samples were collected from all participants to evaluate CXCL3 and leptin serum levels using ELISA. The results demonstrated significantly ($P \leq 0.001$) higher mean levels of CXCL3 and leptin in breast cancer patients (1.19±0.10 ng/mL and 130.44± 3.72pg/mL) compared to those of their healthy counterparts (0.430± 0.02\ng/mL and 57.1± 3.2pg/mL respectively). Interestingly, vast majority of the assessed breast cancer cases (up to 95-98%) showed to have elevated serum levels of both of the assessed potential biomarkers (CXCL3 and leptin). The present study results suggest an association of both CXCL3 and leptin in breast cancer pathogenicity. This supports the possibility of utilizing these potential biomarkers for breast cancer early detection and diagnosis.

Keywords: CXCL3, Leptin, Breast cancer, Diagnostic potential

الدور التشخيصي المحتمل لمستويات CXCL3 و اللبتين في سرطان الثدي

عباس سعد نعمه و فاضل محمد لفتة

قسم علوم الحياة، كلية العلوم ، جامعة بغداد ، بغداد، العراق

الخلاصة

يُعتقد أن خطر الإصابة بسرطان الثدي يُعزى إلى التغيرات في عدد من المكونات البيولوجية الرئيسية. ضمن هذا السياق، ان المستويات المرتفعة لبعض الكيموكينات تعمل كعوامل نمو يمكن أن تعزز تطور السرطان. صممت الدراسة الحالية لتقييم مستوى المصل لـ CXCL3 (كيموكين C–X–C Motif Ligand 3) واللبتين (هرمون ببتيد يتم تصنيعه بواسطة الأنسجة الدهنية ويمتلك نشاط السيتوكين) لمريضات سرطان الثدي العراقيات مقارنة بالنساء السليمات. تضمنت الدراسة 90 مشاركًا، يتألفون من 60 مريضة تم تشخيص إصابتهن بسرطان الثدي و 30 امرأة خالية من المرض وبصحة جيدة ظاهريا كمجموعة مقارنة. تم جمع عينات الدم الوريدي من جميع المشاركات لتقييم مستويات CXCL3 ومصل اللبتين باستخدام تقنيةELISA.أظهرت النتائج وبشكل معنوي ($P \leq 0.001$) مستويات أعلى من CXCL3 واللبتين في مرضى سرطان الثدي (1.19 ± 0.10 نانوغرام / مل

*Email: fadhellafta@sc.uobaghdad.edu.iq

و 130.44 ± 3.72 بيكامول / مل) مقارنة مع نظيراتهن الأصحاء (0.430 ± 0.02 \ ng / مل) و 57.1 ± 3.2 بكامول / مل على التوالي). ومن المثير للاهتمام ، أن الغالبية العظمى من حالات سرطان الثدي التي تم تقييمها (تصل إلى 95–98٪) أظهرت ارتفاع مستويات المصل لكل من المعلمات الحيوية المدروسة (CXCL3 واللبتين).تشير نتائج الدراسة الحالية إلى وجود ارتباط بين كل من CXCL3 واللبتين في إمراضية سرطان الثدي. هذا يدعم إمكانية استخدام هذه المؤشرات الحيوية المحتملة للكشف والتشخيص المبكرعن سرطان الثدي.

Introduction

Breast cancer (BC) is at the top list of malignancies affecting women around the world [1]. Globally, the figure of breast cancer incidence and its related death are predicted to double by 2040 and reach three million new cases and more than one million deaths per year [2, 3]. According to the Iraqi Cancer Registry (ICR), breast cancer accounts for approximately a third of all recorded women malignancies in Iraq [4, 5]. Multiple risk factors are believed to contribute to the initiation and development of breast cancer including genetic alterations, hormonal imbalance, age, use of contraceptives, family history, reproductive history, body mass index, physical activity, exposure to chemical and physical agents etc. [6]. Understanding the underlying biology of the disease could improve patients' stratification may offer novel therapeutic targets and strategies [7, 8]. Detection of cancer at early-stages is key and has significant impact on disease management in respect to the therapeutic options, minimizing short/long-term treatment-associated toxicity, prolong overall survive and improving patients' quality of life [9, 10]. Thus, it is important to explore the utility of breast cancer-associated biological alterations for the development of accurate, efficient and accessible diagnostic approaches, especially in areas with low quality healthcare services, to reduce mortality rates and increase the chances of survival.

C-X-C motif chemokine ligand 3 (CXCL3) is a bioactive small cytokine encoded by human *GRO* gene in chromosome 4. CXCL3 belongs to a unique CXC chemokine family that promotes chemo-attractant in inflammation process and acts as growth factor mediating tumorigenic potential and cancer cell proliferation via the activation of key pathways such as MAPK/ERK [11, 12]. It has been demonstrated that CXCL3 is responsible for regulating monocyte migration and adhesion through the binding of its receptor called CXCR2 on target monocytes' surface [13]. Previous studies have reported high expression of CXCR2 of myeloid-derived suppressor cells (MDSCs, newly identified immature myeloid cells suppress immune responses and expand during cancer). CXCL3 interacts with its receptor that leads to the activation of MDSCs, where the later event inhibits antitumor immunity, thus facilitating tumor progression [14].

Leptin is a polypeptide hormone with a 16 kDa molecular weight and is predominantly produced by adipose tissue and enterocytes in small intestine [15, 16]. This protein is encoded by *LPT* (obesity factor maps to 7q32.1 genomic region) and has principle function in energy balance and regulation of body weight [17]. Besides its role in haematopoiesis, fetal development, immunity, and reproduction [18, 19], circulating leptin promotes triglycerides storage in adipose tissue, it takes a vital role in energy balance [20]. Leptin acts as a growth factor on certain tissues as through the activation of different signaling pathways it increases expression of genes involved in cell cycle regulation such as *CCND1*, via *JAK2-STAT3* pathway, or *VEGFA*, via *MAPK1/3* and *PI3K-AKT1* pathways [21, 22]. It is also demonstrated to have influence in mitogenic activity**,** induction of mitosis in cells, matrix remodeling vascular of endothelial cells by regulating the expression of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) [23]. Of interest, both tissue hyperplasia and regeneration are associated with mitogenesis [24]. The physiological action of leptin is

demonstrated via it's interaction to its receptor (Ob-R) which is primarily found in the brain [25], as well as immune cells, placenta, stomach [26] and other tissues. Overexpression of leptin receptor has been observed in a number of malignancies especially breast cancer [21, 27]. Such effects may be attributed to the influence of leptin and its receptor in promoting cellular proliferation, inducing angiogenesis, and suppressing apoptosis [28, 29]. Leptin is known to induce the production pro-inflammatory mediators such as IL-6, TNF-α, IL-12 via its effects on circulating monocytes, macrophages and endothelial cells [30]. IL-6 also has a pivotal role in the promotion of tumor cells growth. These functions of IL-6 seem to be *STAT3* dependent on a major oncogenic transcription factor that induces up-regulation of target genes responsible for tumour cells survival [31]. In a recent study it has been shown that *IL-6* increases oxidative stress (ROS) and *Rac1* activity [32]. Rac1 protein has multifunction in cells including the control of cell growth and regulation of cytoskeleton [33].

To the best of our knowledge, there is a very limited local data about the association of CXCL3 and leptin in breast cancer. Hence, we probed to assess serum levels of CXCL3 and leptin genes production to investigate their interconnection with the clinicopathological features in Iraqi women with breast cancer.

Subjects and Methods

A total of 90 Iraqi women were enrolled in this study, including 60 women diagnosed with breast cancer who were randomly selected during their attendance at Al-Amal National Hospital for Cancer Management, Baghdad, Iraq. The study extended from August 2022 to March 2023. Ages of breast cancer women ranged between 20-80 years. In addition to 30 apparently healthy women also participated in this study as a control group whose ages ranged between 20-75 years. The study design was approved by research Ethical Committee and Scientific Committee designated by Biology Department, College of Science, University of Baghdad (Ref. CSEC/0922/0081). Venous blood samples (5ml) were drawn from all participants along with clinicopathological (menopausal status, BC stage/grade, ER/PR, HER2 status, therapeutic protocol, contraceptive use, post-traumatic stress, family history, and suppressed anger status) parameters of the investigated breast cancer patients. CXCL3 and leptin serum levels were assessed utilizing human C-X-C motif chemokine 3 (CXCL3) ELISA kit (Cat. No. MBS7219818, MyBioSource / USA) and human leptin ELISA Kit (Cat. No. MBS169298, MyBioSource / USA), according to the manufacturer's instructions.

Statistical analysis was performed using statistical package of social science (SPSS) version 23. The data was expressed as mean ± SD. Statistical comparison between groups was made using t-test and a *P-value* of ≤0.05 was considered significant difference. Differences between observed and expected data of the investigated potential biomarker were assessed using Chi-square test.

Results

Significantly Elevated Level of CXCL3 in the Studied Breast Cancer Patients

The results of the current study showed highly significant (P≤0.001) increase in the CXCL3 serum level in breast cancer patients group (1.191±0.101ng/mL) as compared to the healthy control group (0.430± 0.02 ng/mL). There was 2.6 folds increase in the CXCL3 serum expression levels than that of the healthy counterparts (Figure 1). This suggested that CXCL3 serum level may retain an independent prognostic value apart from other known potential risk-stratification markers.

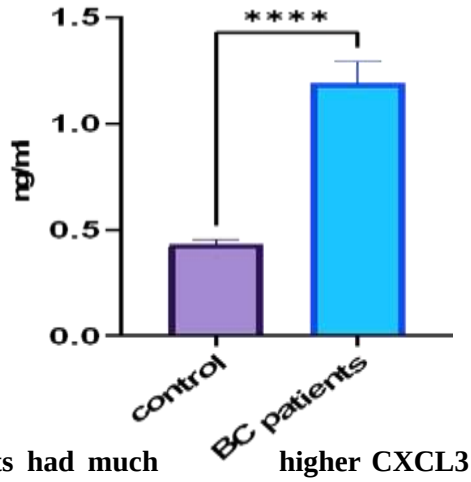

Figure 1: Breast cancer patients had much higher CXCL3 serum level than healthy controls (1.191±0.101 ng/mL and0.430± 0.02 ng/mL respectively, P≤ 0.001).

No significant difference in level of *CXCL3* between breast cancer subgroups was observed according to clinicopathological parameters (Table 1). Additionally, the majority (88%) of Her2-positive breast cancer patients showed lower CXCL3 levels, while only 12% of them exhibited high CXCL3 levels. Regarding the Her2-negative breast cancer patients, significant number of the cases (69%) showed lower levels of CXCL3; whereas only approximately a third of the patients (31%) showed high CXCL3 levels (Figure 2). In triple- positive-breast cancer patients, high percentage of them (90%) showed lower levels of CXCL3 and only 10% presented high CXCL3 levels. While in the triple-negative breast cancer patients, 60% showed lower CXCL3 levels and 40% had high CXCL3 (Figure 3).

Table 1: Statistical relationship between CXCL3 levels and clinicopathology of breast cancer

Clinical Information	CXCL3 (mean ± SD)	P-value
Menopausal status		0.36
Pre	1.05± 0.15	
Post	1.26± 0.13	
HER2		0.14
Positive	0.93± 0.11	
Negative	1.25± 0.19	
TRIPLE		0.14
Positive	3.35± 0.04	
Negative	4.17± 0.93	
Progesterone		0.89
Positive	1.09 ± 0.11	
Negative	1.05± 0.21	
Clinical stages		0. 97
Low stage 1-2	1.21± 0.14 1.21± 0.15	
High stage 3-4		
Post-traumatic stress		0.86
Positive	1.08± 0.11	
Negative	1.12± 0.1	
Suppressed anger status		0.57
Yes	1.25± 0.13	
No	1.13± 0.16	
Contraceptive use		0.96
Yes	1.24± 0.18 1.23± 0.13	
No		
BC family history		0.83
Positive	1.47± 0.18	
Negative	1.53± 0.26	

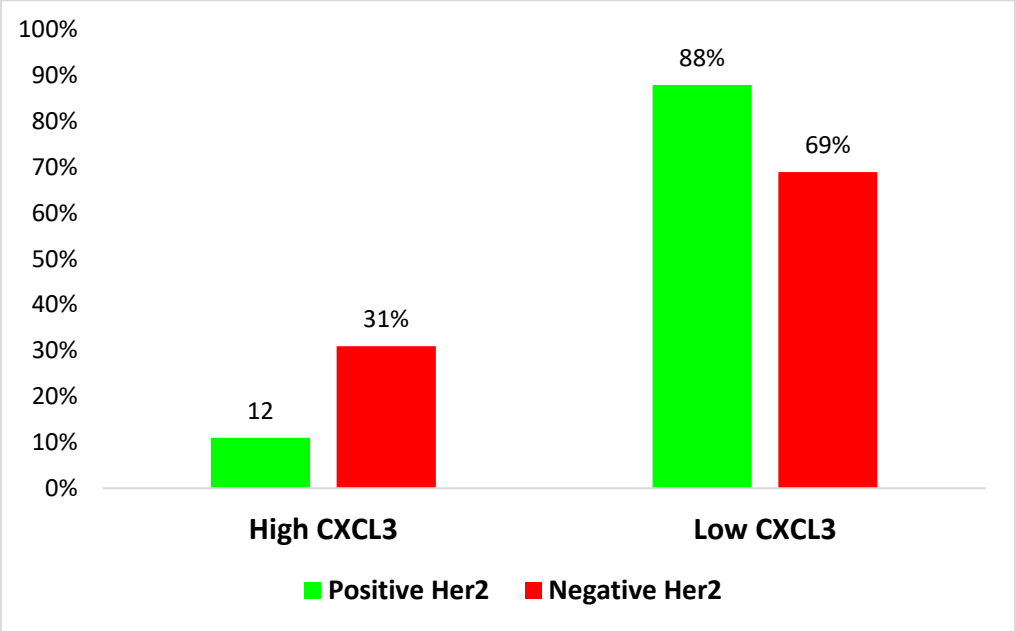

Figure 2: Percentage of high and low observed values of CXCL3 according to HER2 status.

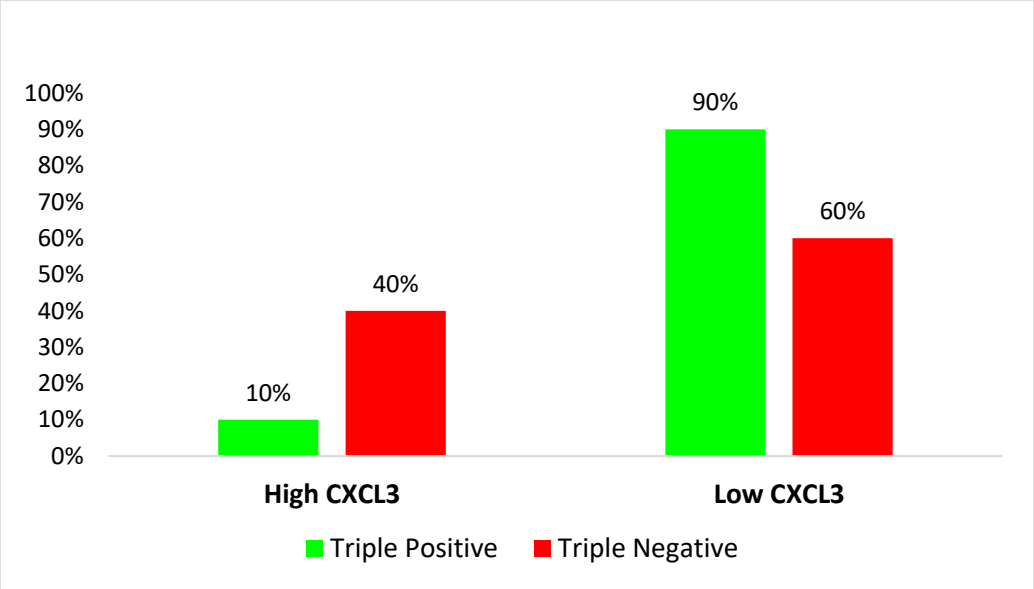

Figure 3: Percentage of high and low observed values of CXCL3 according to hormone receptors status (triple positive or negative).

Leptin Levels in Breast Cancer and Healthy Controls

In respect to the assessment of leptin serum level, breast cancer patients showed to have significantly ($P \leq 0.001$) higher mean levels in comparison to healthy controls (130.44± 3.72 pg/mL and 57.1± 3.2pg/mL respectively, Figure 2). By quantifying the change in leptin serum level, breast cancer patients appeared to have 2.3 folds increase than that of healthy control group.

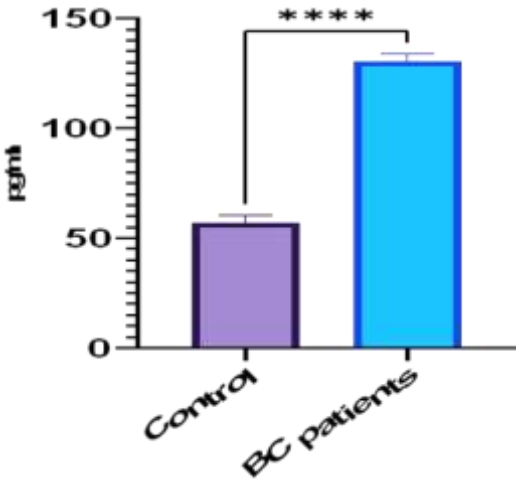

Figure 4: Leptin serum levels in breast cancer and control, data are presented as mean ± SD. Breast cancer patient exhibited 2.3 folds increase in the mean of leptin serum level of than that of healthy counterparts.

Further analysis of the leptin serum levels in relation to the pathoclinical features of the investigated breast cancer patients showed no significant differences among subgroups (Table 2). This suggests that leptin serum level could retain significant prognostic value apart from other known potential risk-stratification markers.

Table 2: Statistical relationship between leptin and clinicopathological and molecular features of breast cancer.

Clinical Information	Leptin (mean±SD)	P-value
Menopausal status		
Pre	129.59± 7.1	0.82
Post	131.48± 4.6	
HER2		
Positive	124± 5.16	0.21
Negative	135.3± 5.84	
TRIPLE		
Positive	125.8± 6.36	0.55
Negative	136.63± 15.91	
Progesterone		
Positive	128.64 ± 4.47	0.97
Negative	128.35± 9.4	
Clinical stages		
Low stage 1-2	129.92 ± 5.75	0. 6
High stage 3-4	134.1 ± 4.95	
Post-traumatic stress		
Positive	125.99± 4.19	0.13
Negative	138.40 ± 7.56	
Suppressed anger status		
Yes	128.09± 4.3	0.73
No	130.62 ± 6.25	
Contraceptive use		
Yes	132.51± 6.75	0.78
No	134.80± 5.18	
BC family history		
Positive	139.77± 5.39	0.37
Negative	148.13± 8.00	

Diagnostic potential role *CXCL3* and leptin diagnostic potential role of CXCL3 and leptin serum levels in breast cancer patients

The study results showed that the vast majoirty of breast cancer patients (95% (57/60)) had high serum levels of CXCL3 in comparison to only (16% (5/30)) of the heathy contorls who showed relatively increased CXCL3 serum levels (Figure 5). These differences resulted in signifcant differences between breast cancer paitients and the healty control group in respect to the CXCL3 serum level status (high or low) significant frequency ($\chi^{2=}$ 57.26, $p < 0.0001$).

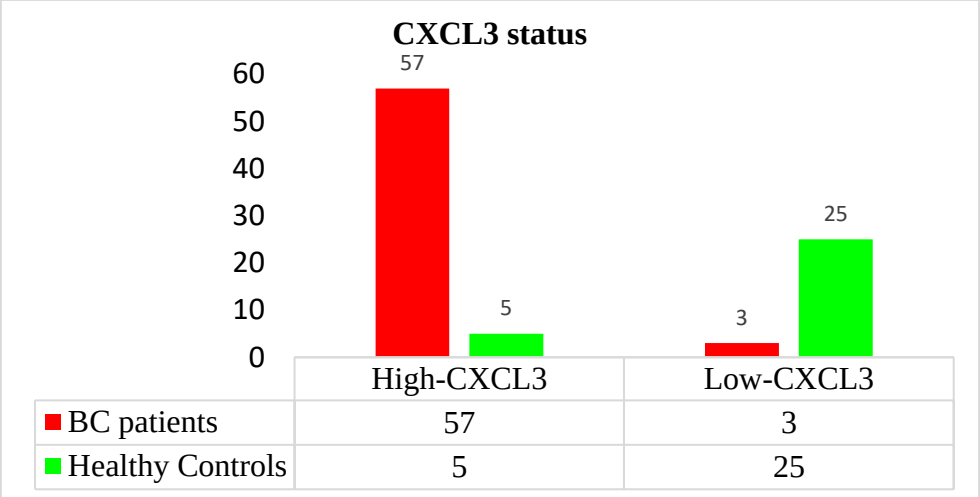

Figure. 5 : Breast cancer patients number according to CXCL3 levels status (high *vs.* low levels).

Similarly, almost all of investigested breast cancer patients (98.33% (59/60)) showed high leptin serum levels. While only one subject from the healthy control group (1/30 (3.33%) showed relatively eleveated leptin serum level (Figure 6). This resulted in signifcant diffrences between breast cancer paitients and the healty control group in respect to the leptin serum level status (high or low).

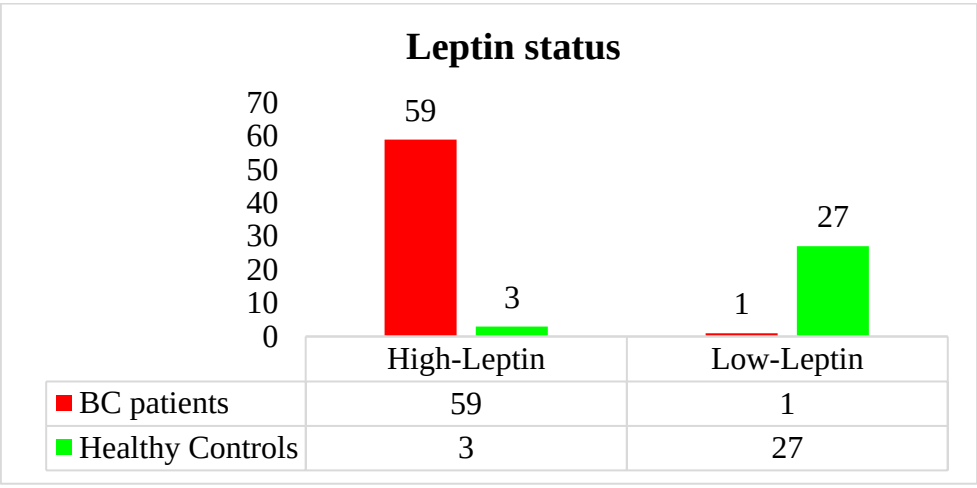

Figure. 6 :Breast cancer patients number according to leptin levels status (high *vs.* low levels).

Collectively, the above presented results suggest that serum level of both CXCL3 and leptin were higher in 95-98.33% of the studied breast cancer patients. This supports the possibity of utilizing these potential biomarkers for breast cancer early detection and diagnosis.

Discussion

Globally, breast cancer is the most common tumor affecting women where its incidence has increased markedly in recent years. Identifying the biological differences between normal and malignant cells is a research priority to understand how cancer initiates and progresses. Thus, the present study was set to investigate the association of CXCL3 and leptin serum level in breast cancer patients. In this study, results showed significantly elevated mean levels of both CXCL3 and leptin in the investigated breast cancer patients in comparison to healthy controls. Interestingly, vast majority of the assessed breast cancer cases (up to 95-98%) showed to have elevated serum levels of both of the assessed potential biomarkers (CXCL3 and leptin). Taken together, these results suggest an impact of CXCL3 and leptin in breast cancer pathogenicity.

Several lines of evidence have supported the association of CXCL3 with tumoergenesis. Of the results that were reported by Ferlay *et. al.* whofound overexpression CXCL3 in the breast and prostate cancer [34]. Similar findings were also observed in cervical cancer [35]. Additionally, CXCL3 proposed to have important role in tumorigenesis and angiogenesis [36]. Cancer associated fibroblast (CAFs), pericytes and tumor cells secrete CXCL3 which performs its functions by binding to C-X-C chemokine receptor type 2 (CXCR2) [37]. CXCR2 expression on different cells including microvascular endothelial cells [38]. Microvascular endothelial cells signficantly effects tumor microenvironment via promoting angiogenesis to sustainable tumor growth and facilities circulating tumor cells through inducting attachment tumor cells to vasicular walls of neighboring tissue [39]. A number of studies have shown that the stromal and epithelial cells in breast cancer (particulary triple negative breast cancer) secrete high levels of soluble factors like CXCL3, besides expressing its receptor CXCR2 [40]. Activation of CXCR2 via CXCL3 promotes proliferation of tumor cells through epidermal growth factor receptors (EGFR) after activation of metalloprotease which cleaves heparin-binding epidermal growth factor-like growth factor (HB-EGF) [41]. CXCR2 activates three principle signaling pathways: the Ras/Raf/extracellular signal related kinases (ERK1/2) pathway, the phospholipase C (PLC)/protein kinase C (PKC) pathway and the phosphatidylinositol-3 kinase (PI3K)/Akt pathway [42]. Activation PI3K/AKT cascade plays a significant role in cell survival, proliferation, migration and invasion of breast cancer [43]. Numerous studies have shown that the over activation of PI3K/AKT pathway reduces apoptosis which allows cells survival, besides proliferation and facilitaes the angiogenesis. Overexpression of CXCL3 can increase the risk of prostate cancer progression and metastasis [44, 45]. In non-small-cell lung cancer (NSCLC), circMET activation proliferation, immune evasion and metastasis all seem to be regulated by Mir-145-5p/CXCL3 [46].

Numerous studies have shown that the leptin promotes inflammatory cytokines, including CXCL3 chemokine in colon cancer [47]. Hence, we suggest that leptin also induces CXCL3 in breast cancer in the same mechanism as in colon cancer. Some studies have showed that leptin levels positively correlate to breast cancer aggressiveness [30]. Leptin has potential role as tumor growth inducible in different cancers, such as pituitary adenomas, cranial tumor [48], through regulation tumor growth and metastatic capacity [49]. A recent study has demonstrated that the leptin could have a potential role in diagnostic of different subtypes of breast cancer [50].

Overall, the present study results have highlight the potential diagnostic value of the evelvated serum levels of CXCL3 and leptin in the studied breast cancer patients. This could be explored further for its relation to different aspects of cancer bioloigy, especially in understanding the mechanisms that underlie fundamental processes such as cell growth, the transformation of normal cells to cancer cells and the spread (metastasis) of cancer cells.

Conclusion

As serum levels of both CXCL3 and leptin signficantly increased in the vast majority of the examined breast cancer patients in comparison to heathy control group, the findings of this study suggest their potential key role in breast cancer pathogencity. Accordnigly, large scale studies are needed to evalute the diagnostic, prognostic and predictive values of these potentiacl biomarkers in breast cancer and other women-affected malignancies.

References

[1] C. Duggan, A. Dvaladze, A. F. Rositch, O. Ginsburg, C. H. Yip, S. Horton, R. Camacho Rodriguez, A. Eniu, M. Mutebi, and J. M. Bourque, "The breast health global initiative 2018 global summit on improving breast healthcare through resource-stratified phased implementation: methods and overview," *Cancer,* vol. 126, pp. 2339-2352, 2020.

[2] H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, and F. Bray, "Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortalityworldwide for 36 cancers in 185 countries," *CA: a cancer journal for clinicians,* vol. 71, pp. 209-249, 2021.

[3] M. Arnold, E. Morgan, H. Rumgay, A. Mafra, D. Singh, M. Laversanne, J. Vignat, J. R. Gralow, F. Cardoso, and S. Siesling, "Current and futureburden of breast cancer: Global statistics for 2020 and 2040," *The Breast,* vol. 66, pp. 15-23, 2022.

[4] N. A. Alwan, D. Kerr, D. Al-Okati, F. Pezella, and F. N. Tawfeeq, "Comparative study on the clinicopathological profiles of breast cancer among Iraqiand British patients," *The Open Public Health Journal,* vol. 11, 2018.

[5] N. A. Alwan, "commentaries Breast Cancer Among Iraqi Women: Preliminary Findings From a Regional Comparative Breast Cancer Research Project," 2016.

[6] S. Łukasiewicz, M. Czeczelewski, A. Forma, J. Baj, R. Sitarz, and A. Stanisławek, "Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies—An Updated Review," *Cancers,* vol. 13, p. 4287, 2021.

[7] C. A. Ali, F. M. Lafta, M. M. AlSayyid, and A.-A. N. G. Al-Rekabi, "BRCA1 Gene Expression is Down Regulated in Both Familial and Sporadic Breast Cancer Cases in Baghdad-Iraq," *Iraqi Journal of Science,* pp. 34-41, 2020.

[8] C. Adel, F. M. Lafta, M. A. Al-Naqqash, and A.-A. N. G. Al-Rekabi, "Loss of the Epigenetically Inactivated-X-Chromosome (Barr Body) a Potential Biomarker for Breast Cancer Development," *Indian Journal of Public Health,* vol. 10, p. 2911, 2019.

[9] L. Wang, "Early diagnosis of breast cancer," *Sensors,* vol. 17, p. 1572.2017 ,

[10] F. Guo, Y. f. Kuo, Y. C. T. Shih, S. H. Giordano, and A. B. Berenson, "Trends in breast cancer mortality by stage at diagnosis among young women in the U nited S tates," *Cancer,* vol. 124, pp. 3500-3509, 2018.

[11] G. T. Ruan, Y. Z. Gong, X. W .Liao, S. Wang, W. Huang, X. K. Wang, G. Z. Zhu, C. Liao, and F. Gao, "Diagnostic and prognostic values of C‐X‐C motif chemokine ligand 3 in patients with colon cancer," *Oncology reports,* vol. 42, pp. 1996-2008, 2019.

[12] Y. L. Qi, Y. Li, X. X. Man, H. Y .Sui, X. L. Zhao, P. X. Zhang, X. S. Qu, H. Zhang, B. X. Wang, and J. Li, "CXCL3 overexpression promotes the tumorigenic potential of uterine cervical cancer cells via the MAPK/ERK pathway," *Journal of cellular physiology,* vol. 235, pp. 4756-4765, 2020.

[13] Y. Cheng, X.-l. Ma, Y.-q. Wei, and X.-W. Wei, "Potential roles and targeted therapy of the CXCLs/CXCR2 axis in cancer and inflammatory diseases," *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer,* vol. 1871, pp. 289-312, 2019.

[14] Y. Cheng, F. Mo, Q. Li, X. Han, H. Shi, S. Chen, Y. Wei, and X. Wei, "Targeting CXCR2 inhibits the progression of lung cancer and promotes therapeutic effect of cisplatin," *Molecular cancer,* vol. 20, pp. 1-21, 2021.

[15] M. Obradovic, E. Sudar-Milovanovic, S. Soskic, M .Essack, S. Arya, A. J. Stewart, T. Gojobori, and E. R. Isenovic, "Leptin and obesity: role and clinical implication," *Frontiers in Endocrinology,* vol. 12, p. 585887, 2021.

[16] I. M. M. Ali, J. H. Yenzeel, and H. M. S. Al-ansari, "Evaluation of oxidative stress and leptinlevel in samples of Iraqi obese women," *Iraqi Journal of Science,* pp. 1565-1570, 2020.

[17] W. F. Zhang, Y. C. Jin, X. M. Li, Z. Yang, D. Wang, and J. J. Cui, "Protective effects of leptin against cerebral ischemia/reperfusion injury ",*Experimental and Therapeutic Medicine*, vol. 17, pp. 3282-3290, 2019.

[18] J. M. Friedman, "Leptin and the endocrine control of energy balance," *Nature Metabolism*, vol. 1, pp. 754-764, 2019.

[19] G. V. Childs, A. K. Odle, M. C. MacNicol, and A. M. MacNicol" ,The importance of leptin to reproduction," *Endocrinology*, vol. 162, p. bqaa204, 2021.

[20] V. Gelen, A. Kükürt, E. Şengül, and H. A. Devecı, "Leptin and Its Role in Oxidative Stress and Apoptosis: An Overview," *Role of Obesity in Human Health and Disease*, p. 143, 2021.

[21] F. Sánchez-Jiménez, A. Pérez-Pérez, L. De la Cruz-Merino, and V. Sánchez-Margalet, "Obesity and breast cancer: role of leptin," *Frontiers in oncology*, vol. 9, p. 596, 2019.

[22] N. K. Saxena, P. M. Vertino, F. A. Anania, and D. Sharma, "Leptin-induced growth stimulation of breast cancer cells involves recruitment of histone acetyltransferases and mediator complex to CYCLIN D1 promoter via activation of Stat3," *Journal of Biological Chemistry*, vol. 282, pp. 13316-13325, 2007.

[23] F .Yu, R. Fu, L. Liu, X. Wang, T. Wu, W. Shen, Z. Gui, X. Mo, B. Fang, and L. Xia, "Leptin-induced angiogenesis of EA. Hy926 endothelial cells via the Akt and Wnt signaling pathways in vitro and in vivo," *Frontiers in Pharmacology*, vol. 10, p. 1275, 2019.

[24] R. Pal, M. A. Kowalik, M. Serra, C. Migliore, S. Giordano, A. Columbano, and A. Perra, "Diverse MicroRNAs-mRNA networks regulate the priming phase of mouse liver regeneration and of direct hyperplasia," *Cell proliferation*, vol. 55, p. e13199, 2022.

[25] K. Glendining, M. Higgins, L. Fisher, and C. Jasoni, "Maternal obesity modulates sexually dimorphic epigenetic regulation and expression of leptin receptor in offspring hippocampus," *Brain, Behavior, and Immunity*, vol. 88, pp. 151-160, 2020.

[26] Y. Kinoshita, S. Arita, T. Ogawa, A. Takenouchi, and K. Inagaki-Ohara, "Augmented leptin-induced trefoil factor 3 expression and epidermal growth factor receptor transactivation differentially influences neoplasia progression in the stomach and colorectum of dietary fat-induced obese mice," *Archives of Biochemistry and Biophysics*, vol. 729, p. 109379, 2022.

[27] L. Gelsomino, G. D. Naimo, R. Malivindi, G. Augimeri, S. Panza, C. Giordano, I. Barone, D. Bonofiglio, L. Mauro, and S. Catalano, "Knockdown of leptinreceptor affects macrophage phenotype in the tumor microenvironment inhibiting breast cancer growth and progression," *Cancers*, vol. 12, p. 2078, 2020.

[28] N. Samad and T. Rao, "Role of leptin in cancer: A systematic review," *Biomed J Sci Tech Res*, vol . ,18pp. 13226-13235, 2019.

[29] S. Nepal, M. J. Kim, J. T. Hong, S. H. Kim, D.-H. Sohn, S. H. Lee, K. Song, D. Y. Choi, E. S. Lee, and P.-H. Park, "Autophagy induction by leptin contributes to suppression of apoptosis in cancer cells and xenograft model :involvement of p53/FoxO3A axis," *Oncotarget*, vol. 6, p. 7166, 2015.

[30] M. F. Atoum, F. Alzoughool, and H. Al-Hourani, "Linkage between obesity leptin and breast cancer," *Breast Cancer: Basic and Clinical Research*, vol. 14, p. 1178223419898458, 2020.

[31] D. C. Chonov, M. M. K. Ignatova, J. R. Ananiev, and M. V. Gulubova, "IL-6 activities in the tumour microenvironment. Part 1," *Open access Macedonian journal of medical sciences*, vol. 7, p. 2391, 2019.

[32] O. Paul, A. Moseley, C. Song, O. O. Al-Khalili ,C. Gomez-Sanchez, D. C. Eaton, and B. M. Wynne, "IL-6 Increases ROS via Rac1, Leading to Mineralocorticoid Receptor and ENaC Activation," *The FASEB Journal*, vol. 34, pp. 1-1, 2020.

[33] L. del Carmen Martínez-Sánchez, P. A. Ngo, R. Pradhan, L.-S. Becker ,D. Boehringer, D. Soteriou, M. Kubankova, C. Schweitzer, T. Koch, and V. Thonn, "Epithelial RAC1-dependent cytoskeleton dynamics controls cell mechanics, cell shedding and barrier integrity in intestinal inflammation," *Gut*, 2022.

[34] L. Zhang, L. Zhang ,H. Li, C. Ge, F. Zhao, H. Tian, T. Chen, G. Jiang, H. Xie, and Y. Cui, "CXCL3 contributes to CD133+ CSCs maintenance and forms a positive feedback regulation loop with CD133 in HCC via Erk1/2 phosphorylation," *Scientific reports*, vol. 6, pp. 1-10, 2016.

[35] J. Guan, J. Weng, Q. Ren, C. Zhang, L. Hu, W. Deng, S. Lu, X. Dong, W. Li, and Y. Li, "Clinical significance and biological functions of chemokine CXCL3 in head and neck squamous cell carcinoma," *Bioscience reports,* vol. 41, p. BSR20212403, 2021.

[36] C. Cui, R. Zhang, F. Gu, Y. Pei, L. Sun, Y. Huang, G. Niu, and J. Li, "Plasma CXCL3 Levels Are Associated with Tumor Progression and an Unfavorable Colorectal Cancer Prognosis," *Journal of Immunology Research,* vol. 2022, 2022.

[37] N. Reyes, S. Figueroa ,R. Tiwari, and J. Geliebter, "CXCL3 signaling in the tumor microenvironment," *Tumor Microenvironment,* pp. 15-24, 2021.

[38] L. Liu, H. Sun, S. Wu, H. Tan, Y. Sun, X. Liu, S. Si, L. Xu, J. Huang, and W. Zhou, "IL‐17A promotes CXCR2‐dependent angiogenesisin a mouse model of liver cancer," *Molecular Medicine Reports,* vol. 20, pp. 1065-1074, 2019.

[39] T. Toya, J. D. Sara, M. T. Corban, R. Taher, S. Godo, J. Herrmann, L. O. Lerman, and A. Lerman, "Assessment of peripheral endothelial function predicts future risk of solid-tumor cancer," *European journal of preventive cardiology,* vol. 27, pp. 608-618, 2020.

[40] F. Boissière-Michot, W. Jacot, J. Fraisse, S. Gourgou, C. Timaxian, and G. Lazennec, "Prognostic value of CXCR2 in breast cancer," *Cancers,* vol. 12 ,p. 2076, 2020.

[41] C. Bolitho, M. A. Hahn, R. C. Baxter, and D. J. Marsh, "The chemokine CXCL1 induces proliferation in epithelial ovarian cancer cells by transactivation of the epidermal growth factor receptor," *Endocrine-related cancer,* vol. 17, pp.2010 ,940-929 .

[42] F. Guo, L. Long, J. Wang, Y. Wang, Y. Liu, L. Wang, and F. Luo, "Insights on CXC chemokine receptor 2 in breast cancer: An emerging target for oncotherapy," *Oncology letters,* vol. 18, pp. 5699-5708, 2019.

[43] D. Miricescu, A. Totan, I-.I. Stanescu-Spinu, S. C. Badoiu, C. Stefani, and M. Greabu, "PI3K/AKT/mTOR signaling pathway in breast cancer: From molecular landscape to clinical aspects," *International journal of molecular sciences,* vol. 22, p. 173, 2020.

[44] S.-l. Gui, L.-c. Teng ,S.-q. Wang, S. Liu, Y.-L. Lin, X.-l. Zhao, L. Liu, H.-y. Sui, Y. Yang, and L.-c. Liang, "Overexpression of CXCL3 can enhance the oncogenic potential of prostate cancer," *International urology and nephrology,* vol. 48, pp. 701-709, 2016.

[45] A. Samakova ,A. Gazova, N. Sabova, S. Valaskova, M. Jurikova, and J. Kyselovic, "The PI3k/Akt pathway is associated with angiogenesis, oxidative stress and survival of mesenchymal stem cells in pathophysiologic condition in ischemia," *Physiological research,* vol. 68 ,pp. S131-S138, 2019.

[46] X. Pei, S.-W. Chen, X. Long, S.-Q. Zhu, B.-Q. Qiu, K. Lin, F. Lu, J.-J. Xu, P.-F. Zhang, and Y.-B. Wu, "circMET promotes NSCLC cell proliferation, metastasis, and immune evasion by regulating the miR-145-5p/CXCL3 axis," *Aging (Albany NY),* vol. 12, p. 13038, 2020.

[47] A. J. Farquharson, R. J. Steele, F. A. Carey, and J. E. Drew, "Novel multiplex method to assess insulin, leptin and adiponectin regulation of inflammatory cytokines associated with colon cancer," *Molecular biology reports,* vol. 39, pp. 5727-5736, 2012.

[48] T. M. Kinfe, A. Stadlbauer, Y. Bozhkov, N. Kremenevski, S. Brandner, M. Buchfelder, and S. R. Chaudhry, "The diagnostic and therapeutic role of leptin and its receptor ObR in glioblastoma multiforme," *Cancers,* vol. 12, p. 3691, 2020.

[49] I. Haque, A. Ghosh, S. Acup, S. Banerjee, K. Dhar, A. Ray, S. Sarkar, S. Kambhampati, and S. K. Banerjee, "Leptin-induced ER-α-positive breast cancer cell viability and migration is mediated by suppressing CCN5-signaling via activating JAK/AKT/STAT-pathway," *BMC cancer,* vol. 18, pp. 1-14, 2018.

[50] L. García-Estevez, S. González-Martínez, and G. Moreno-Bueno, "The leptin axis and its association with the adaptive immune system in breast cancer," *Frontiers in Immunology,* vol. 12 , .2021