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Diagnostic Potential Role of CXCL3 and Leptin Levels in Breast Cancer

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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 ± 3.72 pg/mL) compared to those of their healthy counterparts (0.430 ± 0.02 ng/mL and 57.1 ± 3.2 pg/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، إن المستويات المرتفعة لبعض الكيموكينات التي 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) لمرضى سرطان الثدي العراقيين مقارنة بالنساء السليمات. تضمنت الدراسة ما مجموعه 90 مشاركاً، يتألفون من 60 مريضة تم تشخيص إصابتهن بسرطان الثدي و 30 امرأة خالية من المرض وبصحة جيدة ظاهرياً كمجموعة مقارنة. تم جمع عينات الدم الوريدي من جميع المشاركات لتقييم مستويات CXCL3 ومصل اللبتين باستخدام

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تقنية ELISA. أظهرت النتائج وبشكل معنوي ($P \leq 0.001$) مستويات أعلى من CXCL3 واللبتين في مرضى سرطان الثدي (0.10 ± 1.19 نانوغرام / مل و 3.72 ± 130.44 بيكامول / مل) مقارنة مع نظيرائهن الأصحاء (0.02 ± 0.430 ng / مل) و 3.2 ± 57.1 بيكامول / 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 المحتملة التي تم تقييمها (CXCL3 واللبتين). تشير نتائج الدراسة الحالية إلى وجود ارتباط بين كل من CXCL3 واللبتين في إمرضية سرطان الثدي. هذا يدعم إمكانية استخدام هذه المؤشرات الحيوية المحتملة للكشف والتشخيص المبكر عن 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 its 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 (45) years. In addition to 30 apparently healthy women also participated in this study as a control group whose ages ranged between 20-75 (40) 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 \pm 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 ($P \leq 0.001$) increase in the CXCL3 serum level in breast cancer patients group (1.191 ± 0.101 ng/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.

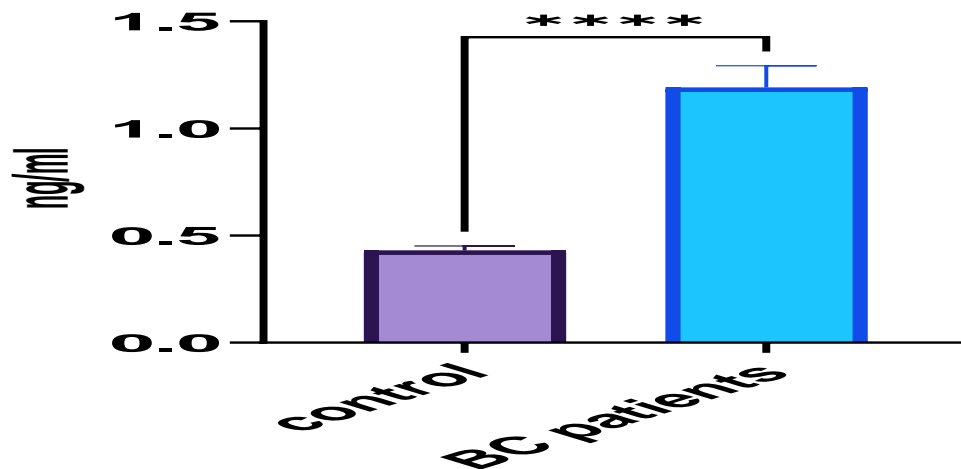


Figure 1: Serum levels of CXCL3 in breast cancer and healthy control. Data is presented as mean \pm SD; breast cancer patients had much higher CXCL3 serum level than healthy controls (1.191 ± 0.101 ng/mL and 0.430 ± 0.02 ng/mL respectively, $P \leq 0.001$).

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 serum levels and clinicopathology of breast cancer

Clinical Information	CXCL3 Value	p value	Clinical Information	CXCL3 Value	p value
Menopausal Status			Clinical Stage		
pre	1.05± 0.15	0.36	low stage 1-2	1.21± 0.14	0.97
post	1.26± 0.13		high stage 3-4	1.21± 0.15	
HER2			Suppressed Anger Status		
Positive	0.93± 0.11	0.14	Positive	1.25± 0.13	0.57
Negative	1.25± 0.19		Negative	1.13± 0.16	
TRIPLE			Contraceptive		
Positive	0.85± 0.09	0.14	Use	1.24± 0.18	0.96
Negative	1.30± 0.38		Non-use	1.23± 0.13	
Progesterone			Family History		
Positive	1.09 ± 0.11	0.89	Positive	1.47± 0.18	0.83
Negative	1.05± 0.21		Negative	1.53± 0.26	
Post-traumatic Stress					
Positive	1.08± 0.11	0.86			
Negative	1.12± 0.1				

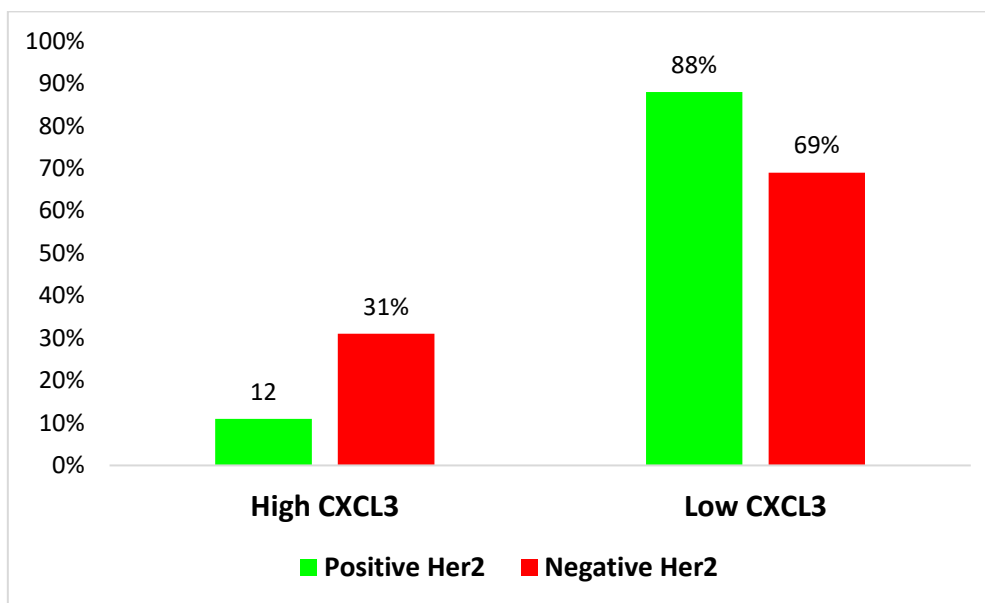


Figure 2: Percentage of high and low observed values of CXCL3 according to HER2 status.

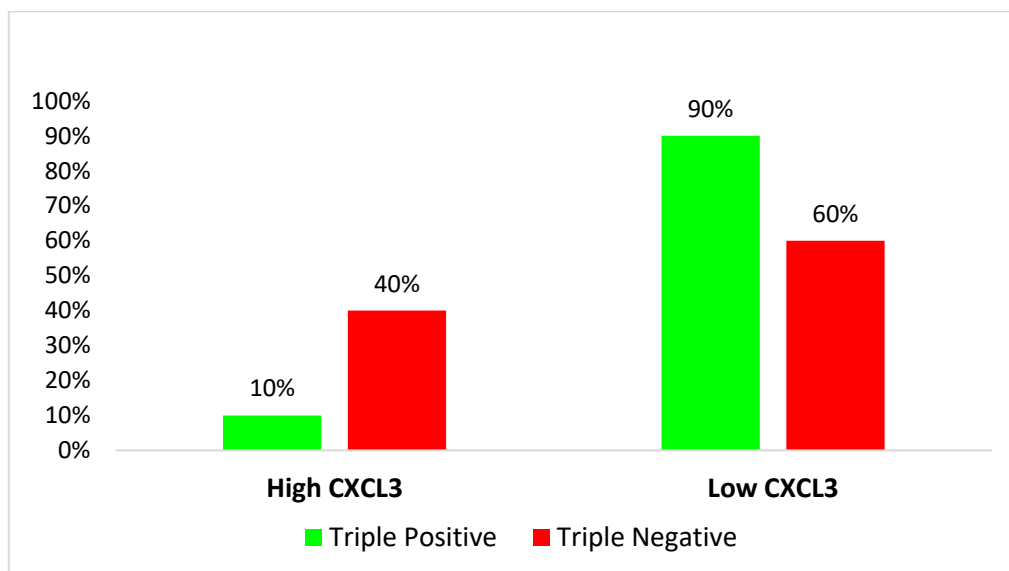


Figure 3: Percentage of high and low observed values of CXCL3 according to hormone receptors status (triple positive or negative).

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 ± 3.2 pg/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.

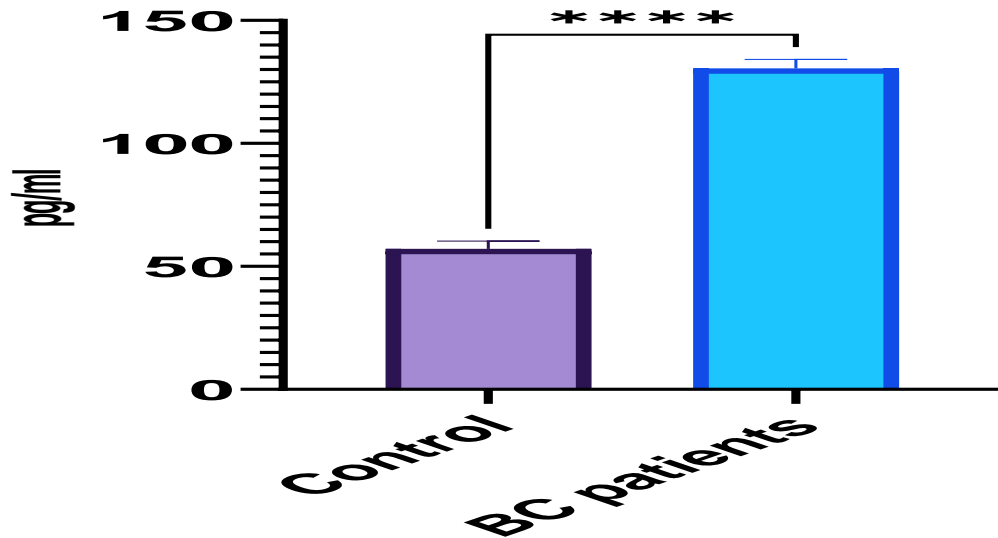


Figure 4: Leptin serum levels in breast cancer and control, data are presented as mean \pm SD. Breast cancer patient exhibited 2.3 folds increase in the mean of leptin serum level of than that of healthy counterparts.

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 value	p value	Clinical Information	Leptin value	p value
Menopausal status			Clinical Stage		
Pre	129.59± 7.1	0.82	Low stages 1-2	129.92±5.75	0.6
Post	131.48± 4.6		High stages3-4	134.1±4.95	
HER2			Suppressed Anger Status		
Positive	124± 5.16	0.21	Positive	128.09±4.3	0.73
Negative	135.3±5.84		Negative	130.62± 6.25	
TRIPLE			Contraceptive		
Positive	125.8± 6.36	0.55	Use	132.51±6.75	0.78
Negative	136.63±15.91		Non-use	134.8±5.18	
PR			Family History		
Positive	128.64 ± 4.47	0.97	Positive	139.77±5.39	0.37
Negative	128.35 ± 9.4		Negative	148.13± 8.0	
Post-traumatic Stress					
Positive	125.99±4.19	0.13			
Negative	138.4±7.56				

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 majority of breast cancer patients (95% (57/60)) had high serum levels of CXCL3 in comparison to only (16% (5/30)) of the healthy controls who showed relatively increased CXCL3 serum levels (Figure 5). These differences resulted in significant differences between breast cancer patients and the healthy control group in respect to the CXCL3 serum level status (high or low) significant frequency ($\chi^2=57.26$, $p < 0.0001$).

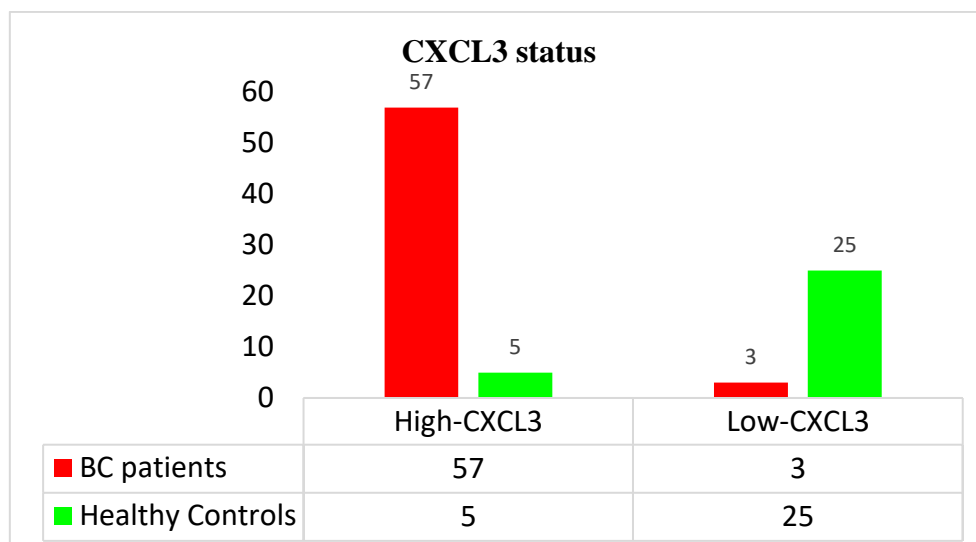


Figure. 5 : The number breast cancer patients according to the CXCL3 levels status (high or low levels).

Similarly, almost all of investigated 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 elevated leptin serum level (Figure 6). This resulted in significant differences between breast cancer patients and the healthy control group in respect to the leptin serum level status (high or low).

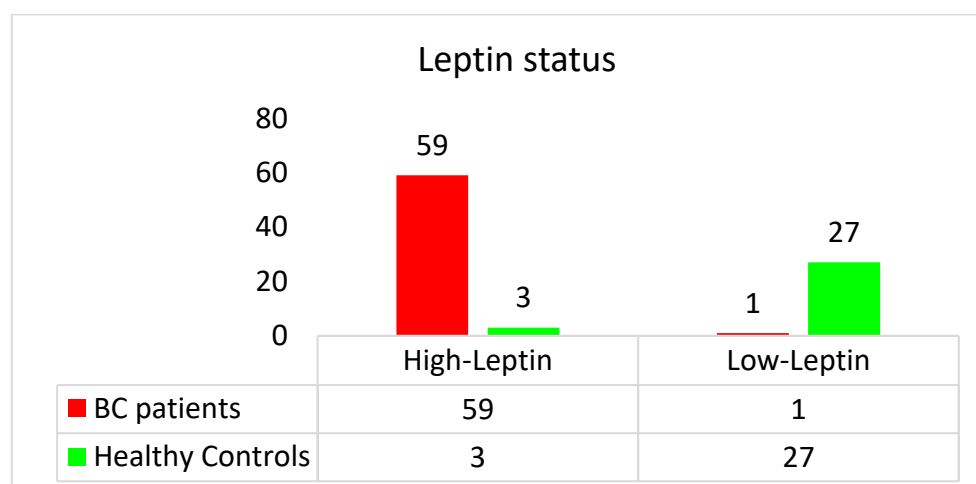


Figure. 6 : The number breast cancer patients according to the leptin levels status (high or low levels).

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

possibility 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 tumorigenesis. Of the results that were reported by Ferlay *et al.* who found 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 significantly effects tumor microenvironment via promoting angiogenesis to sustainable tumor growth and facilitates circulating tumor cells through inducing attachment tumor cells to vascular walls of neighboring tissue [39]. A number of studies have shown that the stromal and epithelial cells in breast cancer (particularly 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 facilitates 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 elevated 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 biology, 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 significantly increased in the vast majority of the examined breast cancer patients in comparison to healthy control group, the findings of this study suggest their potential key role in breast cancer pathogenicity. Accordingly, large scale studies are needed to evaluate the diagnostic, prognostic and predictive values of these potential biomarkers in breast cancer and other women-affected malignancies.

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