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Serum CCL2 and CXCL8 Levels in Breast Cancer Patients Undergoing Treatment in Karbala, Iraq: Associations with Hormonal Receptor Status and Therapeutic Modalities

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Abstract

Background: Breast cancer is among the most prevalent cancers amongst females and is strongly related to inflammatory reactions in the tumor microenvironment. The role of chemokines such as CCL2 and CXCL8 in the development of tumor cells, tumor angiogenesis, and immune system modulation is well established. **objective:** The current study aimed to assess serum concentrations of CCL2 and CXCL8 chemokines in breast cancer patients under treatment in Karbala governorate, Iraq, and the correlation with hormonal receptor HER2, ER, PR status and treatment strategies. **Methodology:** A case-control design was carried out on 60 breast cancer patients and 40 healthy subjects. Blood samples were taken from patients during their treatment, and full blood count was conducted. Measurement of serum concentrations of CCL2 and CXCL8 was done using sandwich ELISA method. Statistical analysis was performed using SPSS program version 25, and $p \leq 0.05$ was considered statistically significant. **Results:** The outcomes indicate that there was a remarkable difference in chemokine serum concentrations between breast cancer patients and controls. Serum concentration of CCL2 was considerably lower than that in the control group in some molecular subtypes while the serum concentration of CXCL8 was significantly higher in breast cancer patients ($p = 0.012$). There was a significant association between chemokine concentration and HER2, ER, and PR receptor status. Significantly higher concentrations of CCL2 and CXCL8 were also highly correlated with the treatment

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strategy, with the maximum concentration being observed in those treated with chemotherapy and radiotherapy ($p \leq 0.001$). High positive correlations between CCL2 and CXCL8 were established. The above findings show that CCL2 and CXCL8 have an important role in the inflammatory microenvironment of breast cancer. CXCL8 concentration is constantly higher in breast cancer patients while CCL2 concentration varies according to the receptor status and treatment type. **Conclusions:** CXCL8 may be used as a stable biomarker for systemic inflammation in breast cancer patients undergoing treatment. CCL2 levels are affected by hormone receptor status and the type of treatment. Further longitudinal studies are needed to evaluate its diagnostic and prognostic potential.

Keywords: Breast cancer, CCL2, CXCL8, ELISA, HER2, ER, PR, Inflammation, Iraq

Introduction

Breast cancer still occupies the top rank as the most prevalent form of cancer in women across the world, imposing an important public health problem [1]. In addition to genetic changes, tumor development is also regulated by the tumor microenvironment, especially the inflammatory cytokines, for example, chemokines [2]. Chemokines are small cytokines responsible for the recruitment of immune cells and modulation of inflammatory processes. Some of these include C-C motif chemokine ligand 2 (CCL2) and C-X-C motif chemokine ligand 8 (CXCL8), which have important functions in tumor development, angiogenesis, metastasis, and immune response [3]. CCL2 was shown to be able to induce the recruitment of monocytes and macrophages to tumor sites, leading to tumor inflammation and tumor development [4]. On the other hand, CXCL8 is a powerful pro-inflammatory chemokine capable of promoting angiogenesis and tumor cell motility [5]. Despite the accumulation of data globally, very little information is available on the circulating levels of CCL2 and CXCL8 in Iraqi breast cancer patients, especially those receiving treatments. Thus, this current study was designed to determine the levels of circulating CCL2 and CXCL8 in Iraqi breast cancer patients under treatment in Karbala governorate, Iraq.

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Material and Method

Experimental Design

This A **cross-sectional case-control study** was conducted at the Imam Hussein (AS) Center for Oncology and Hematology in the holy city of Karbala from November 2024 to July 2025. The study included 100 women, sixty women diagnosed with breast cancer and receiving treatment (chemotherapy, radiotherapy, or biological therapy) were enrolled, alongside forty age-matched healthy women as controls. The study utilized risk factors and measured the concentration levels of the immunological markers CCL2 and CXCL8. It also investigated the relationship between HER2 levels and the risk factors associated with elevated CCL2 and CXCL8 immunological markers.

Collection of Blood Samples

Two ml of venous blood were withdrawn from women with breast cancer using medical syringes. The blood was transferred to gel tubes and left at room temperature for 30 minutes. The serum was then centrifuged and separated, and placed in Eppendorf tubes. It was stored at -20°C until use. All samples were identified by a serial number and the patient's name.

Interleukin Immunoassay through ELISA:

Measurement of the human CCL2 / CXCL8 chemokine was done via a sandwich ELISA method according to the procedure outlined by the company's instruction. This was done as described below:

1. Preparation of Reagents, Standards, and Samples: All reagents, standards, and samples were prepared according to the instructions supplied with the kit.All components were allowed to reach room temperature before starting the analysis, and all steps were performed at room temperature.

2. Preparation of the ELISA Plate: The required number of strips was determined and inserted into the appropriate frame. Unused strips were stored at 2–8°C to ensure their safety.

3. Loading Samples and Standards: 50 µL of the standard solution was loaded into its respective wells (without adding an antibody, as the solution already contained the

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biotin antibody). 40 µL of the sample was added to the sample wells, followed by 10 µL of human antibody CCL2\ CXCL8

50 µL of Streptavidin-HRP solution was then added to the sample and control wells (excluding the empty control well). The contents were gently mixed, the plate was covered, and incubated for 60 minutes at 37°C.

4. Plate Washing: The cover was removed, and the wells were washed five times with the wash solution During each wash, the wells were immersed in 300 µL of the solution for 30 seconds to 1 minute, then aspirated and dried using absorbent wipes.

5. Substrate Reaction:50 µL of Substrate A solution and then 50 µL of Substrate B were added to each well. The plate was incubated for 10 minutes in the dark at 37°C after being re-covered.

6. Stopping the reaction and reading the results:50 µL of stop solution was added, causing the color to change from blue to yellow.

Optical absorbance (OD) was measured directly using an ELISA reader at a wavelength of 450 nm within 10 minutes of adding the stop solution.

Statistical Analysis:

Analyses were performed using SPSS version 25. Data distribution was assessed using the Shapiro-Wilk test. Non-normally distributed data were reported as the mean (interquartile range). Group comparisons were performed using the Mann-Whitney U test or the Kruskal-Wallis test with post-hoc Dunne adjustment. Correlations between CCL2 and CXCL8 within individuals were analyzed using Spearman's rank correlation coefficient. Correlations between chemokine levels, receptor status, and treatment modality were assessed using multivariate regression models adjusted for age, body mass index, menopausal status, and white blood cell count. A p-value ≤ 0.05 was considered statistically significant.

Ethical Approval

The study protocol was approved by the Ethics Committee of the Ministry of Health in Karbala Governorate. A research project consent form was completed at the Ministry of Health (Form No. 2024/Issue 3783). Informed consent was obtained from all study participants in the form of a questionnaire.

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Results and Discussion

The relationship between CCL2/CXCL8 chemokine levels and HER2 status in breast cancer patients and non-hereditary women, and their inflammatory and pathological implications.

Table 1 shows significant differences in the mean concentrations of CCL2 and CXCL8 chemokines between the control group and breast cancer patients classified according to HER2 status. The mean CCL2 concentration was higher in the control group (0.4572 ± 0.1705) compared to HER2-positive patients (0.08695 ± 0.0724) and HER2-negative patients (0.06329 ± 0.09432), with a very high statistical significance ($p = 0.0001$). For CXCL8, the control group recorded a mean of (0.9020 ± 0.2728), while the mean increased to (1.0123 ± 0.0953) in HER2-positive patients and to (1.1071 ± 0.1073) in HER2-negative patients. The difference between the groups was statistically significant ($p = 0.012$).

Table (1) Comparison of mean CCL2 and CXCL8 concentrations between the control group and breast cancer patients as measured by HER2

Variables	Parameters	Mean ± SD	p-value	LDS value
CCL2	Control	0.1705 ± 0.4572	0.0001	0.138
	Positive Her2	0.0734 ± 0.8695		
	negative Her2	0.0943 ± 0.6939		
CXCL8	Control	0.9020 ± 0.2728	0.012	0.186
	Positive Her2	1.0123 ± 0.0953		
	negative Her2	1.1071 ± 0.1073		

As shown in Table (2) and Figure (1), the correlation coefficients for CCL2 levels demonstrated a negative correlation between the control group and HER2-positive patients ($r = -0.431$) and between the control group and HER2-negative patients ($r = -0.288$). Conversely, a strong positive correlation was observed between the HER2-positive and HER2-negative groups ($r = +0.648$), indicating a similar pattern of CCL2 change within the patient groups despite differences in HER2 status. Regarding CXCL8.

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Table (2) Pearson Correlation (r) coefficient for CCL2 level between different groups in terms of HER2

The relationship between the groups CCL2	Correlation coefficient (r)	Statistical interpretation
Control and HER2 positive	r =- 0.431	Weak negative (reverse) correlation
Control and HER2 negative	r =-0.288	Weak (negative) correlation
HER2 positive and HER2 negative	r = 0.648	Strong positive correlation (direct)

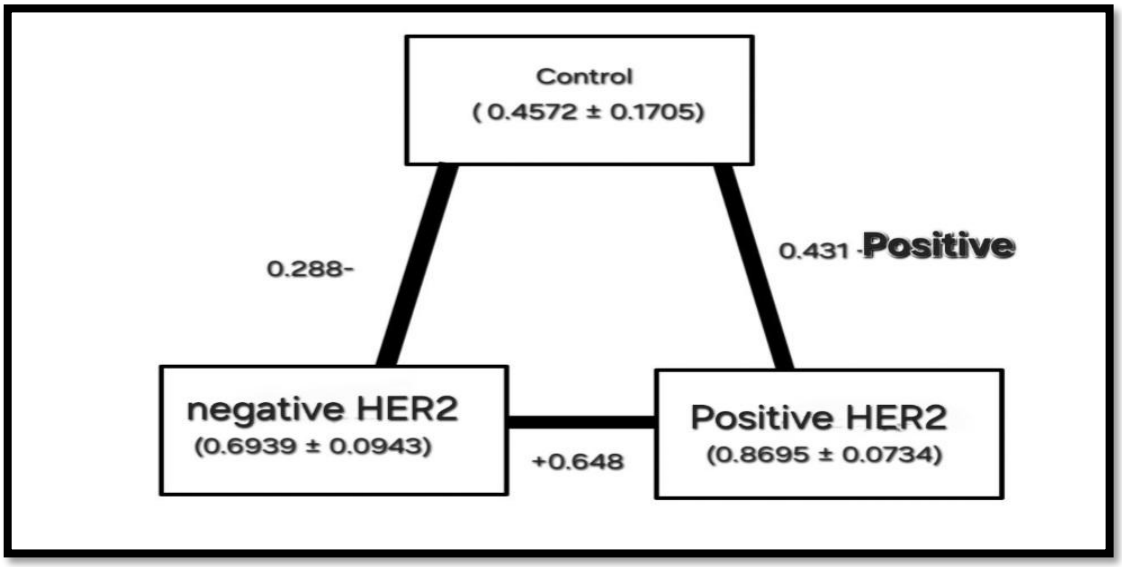

Figure (1) is a diagram illustrating the relationships between the three groups of chemokines CCL2

Table (3) and Figure (2) showed a negative correlation between the control group and HER2-positive patients (r = −0.47) and between the control group and HER2-negative patients (r = −0.19). A moderate positive correlation was also observed between the HER2-positive and HER2-negative groups (r = +0.52), suggesting that CXCL8 acts as a common inflammatory marker across the patient groups, albeit with varying degrees of elevation.

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Table (3) Pearson Correlation (r) coefficient for CXCL8 level between different groups as a function of HER2

The relationship between the CXCL8 groups	Correlation coefficient (r)	Statistical interpretation
Control and HER2 positive	r = -0.47	Inverse weak correlation
Control and HER2 negative	r = -0.19	Weak inverse correlation
HER2 positive and HER2 negative	r = +0.52	The correlation is a positive mean.

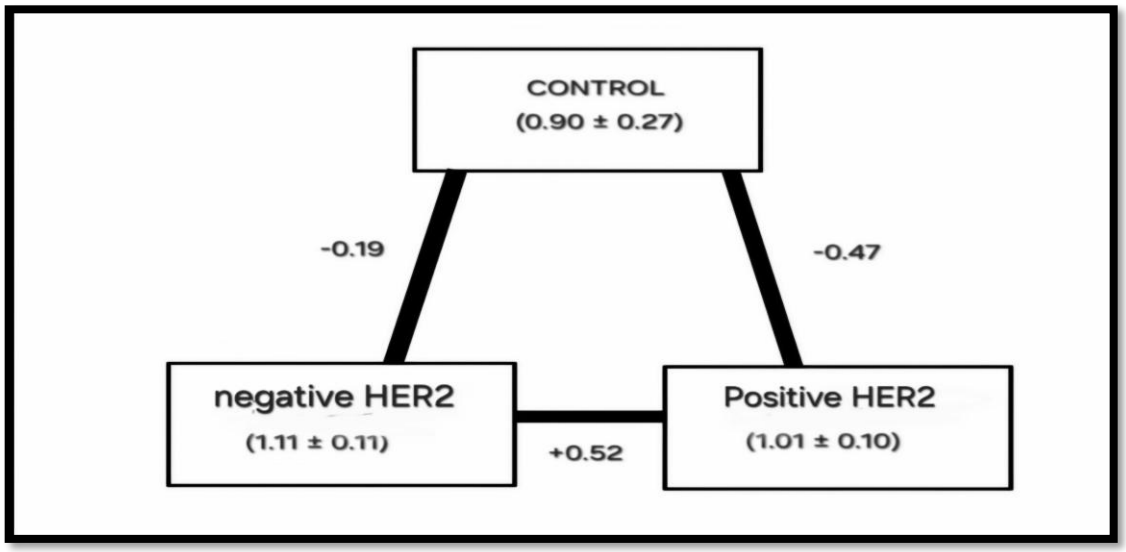

Figure (2) is a diagram illustrating the relationships between the three chemokine groups .CXCL8

The current study shows significant differences in serum levels of the chemokines CCL2 and CXCL8 (IL-8) between the control group and breast cancer patients classified according to HER2 status. As for CCL2, the mean concentration of the biomarker was significantly higher in the control group compared to patients categorized as HER2 positive and HER2 negative (p = 0.0001), namely, ≈0.4572 ± 0.1705 in the controls, and ≈0.0869 ± 0.0724 in HER2 positive, and ≈0.0633 ± 0.0943 in HER2 negative. The reduction in the concentration of CCL2 in patients compared to healthy subjects indicates a trend opposite to numerous research findings, which demonstrated increased concentrations of CCL2 in the blood plasma of patients suffering from breast cancer and in the tumor body. This suggests that the local expression of CCL2 in the tumor body might not necessarily correlate with the concentration in the serum; the difference could be attributed to the effect of the

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immune system and chemotherapy/radiotherapy. This study refutes the views of the researcher [6]. In the tumor inflammatory microenvironment, their research found that the CCL2 protein is highly expressed in breast cancer tissues and recruits TAMs and drives tumorigenesis. Increased expression can be explained by numerous tumor variants. Furthermore, a broader analysis of CCL2 expression in different cancer tissues demonstrated that high CCL2 expression in tumor tissue is linked to genetic molecular alterations and its interactions with hormone receptors. This contrasts with the low serum CCL2 levels observed in our study [7] Additionally, another study found that CCL2 and CCL5 are highly expressed at tumor sites and that they increase TAM presence and promote angiogenesis, migration, and metastasis in breast cancer [8]. The low serum CCL2 levels observed in our study are explained by the circulating blood levels. It may decrease after treatment or as a result of chemokine redistribution within the tumor and its surrounding immune environment. Furthermore, circulating blood CCL2 concentration does not always reflect intratumorally expression, particularly in the presence of systemic immune signaling or treatment-induced inflammatory changes.

As for CXCL8 (IL-8), a significant increase in its levels was observed in patients compared to the control group ($p = 0.012$), with the highest levels in HER2-negative patients ($\approx 1.1071 \pm 0.1073$) followed by HER2-positive patients ($\approx 1.0123 \pm 0.0953$), compared to the control group ($\approx 0.9020 \pm 0.2728$). This observation corresponds to the literature on the connection of IL-8 involvement in inflammation processes, promotion of angiogenesis and tumor cells migration and metastasis development in breast cancer. The CXCL8-CXCR1/2 axis initiates the signaling cascade that influences cell proliferation, differentiation, migration and is recognized as an inflammatory marker connected with the disease development. [9].

The result of correlation analysis between groups showed that negative correlations between control and patient groups for both CCL2 and CXCL8 mean that the change from normal to pathological condition is connected with general reduction of circulating CCL2 expression level in comparison with control group. Elevated CXCL8 expression, in turn, correlates positively with the development of the disease in patients because of increased activity of the inflammation signaling pathways in comparison with control group patients. Positive correlation between both markers in both HER2-positive and HER2-negative patients shows the similarity of inflammatory processes in

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different types of the disease. In general, one can note that CCL2 and CXCL8 differ in behavior in circulating blood of patients with breast cancer, namely, CCL2 is relatively decreased while CXCL8 increases.

The Effect of Estrogen Receptor (ER) Status on CCL2 and CXCL8 Chemokines and Their Relationship to the Inflammatory Response in Breast Cancer Patients

Table 4 shows a significant increase in the mean concentration of the chemokine CCL2 in breast cancer patients compared to the control group. The mean CCL2 concentration in the control group was 0.4421 ± 0.1813, while it increased to 0.7301 ± 0.1233 in the ER-negative group and to 0.6865 ± 0.0899 in the ER-positive group. The results also showed a significant difference between the groups at a significance level of $p = 0.014$, indicating that estrogen receptor status is associated with increased CCL2 levels in patients compared to non-patients. Regarding the chemokine CXCL8, the results also showed an increase in its level in breast cancer patients compared to the control group. The mean CXCL8 in the control group was 0.8890 ± 0.2486, while it increased to 1.1104 ± 0.1323 in the ER-negative group and to 1.0926 ± 0.1233 in the ER-positive group. These differences were statistically significant at $p = 0.027$, confirming the association of breast cancer with elevated CXCL8 as a clear inflammatory marker.

Table (4) Comparison of mean CCL2 and CXCL8 concentrations between the control group and breast cancer patients as indicated by estrogen receptor (ER)

Variables	Parameters	Mean ± SD	p-value	LDS value
CCL2	Control	0.1813 ± 0.4421	0.014	0.174
	negative ER	0.1233 ± 0.7301		
	Positive ER	0.0943 ± 0.6939		
CXCL8	Control	0.2486 ± 0.8890	0.027	0.224
	negative ER	0.1323 ± 1.1104		
	Positive ER	0.1233 ± 1.0926		

The results in Table (5) show that the Pearson correlation coefficient ® for CCL2 levels between the control group and the ER-negative group was r = 0.64. This correlation is described as moderate to strong, indicating a similar pattern of change in

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CCL2 levels between the control and ER-negative groups, but with a marked increase in the patients. The results also showed a moderate correlation between the control group and the ER-positive group, with a correlation coefficient of r = 0.52, indicating that CCL2 also increases in ER-positive groups, but to a lesser extent compared to ER-negative groups. A moderate to weak correlation was found between the ER-negative and ER-positive groups, with a value of r = 0.38, suggesting a relative difference in the pattern of CCL2 levels between these two groups. This may reflect differences in tumor biology and the severity of the inflammatory response depending on the ER status.

Table (5) Pearson Correlation (r) for CCL2 levels between different groups as a function of estrogen receptor (ER)

The relationship between the groups CCL2	Correlation coefficient (r)	Statistical interpretation
Control & ER Negative	**r =0.64**	**Moderate to strong correlation**
Control and ER positive	**r =0.52**	**Moderate correlation**
ER negative and ER positive	**r = 0.38**	**weak to moderate correlation**

Figure (3) illustrates the general trend of the relationships between the three groups (control, ER positive, and ER negative) with respect to CCL2 levels. It clearly shows that the highest CCL2 levels were found among the patient groups compared to the control group, with a relative prominence in the ER negative group. The figure also supports the results of Table (5), which demonstrated a stronger correlation between control and ER negative compared to the correlation between control and ER positive. The results in Table (6) show that the Pearson correlation coefficient ® for CXCL8 levels between the control group and the ER-negative group was r = 0.405, a weak to moderate correlation. This indicates that the pattern of CXCL8 change between the control and ER-negative groups is less homogeneous compared to what was observed in CCL2. In contrast, a moderate to strong correlation was found between the control group and the ER-positive group, with a value of r = 0.622, which is higher than the correlation value with ER-negative. This may suggest that CXCL8 in ER-positive groups follows a pattern closer to the control group in terms of relative distribution, despite its higher value. The results also showed a moderate correlation between the

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ER-negative and ER-positive groups, with a correlation coefficient of r = 0.537. This indicates a relative convergence in CXCL8 levels between the patient groups, but not a complete match.

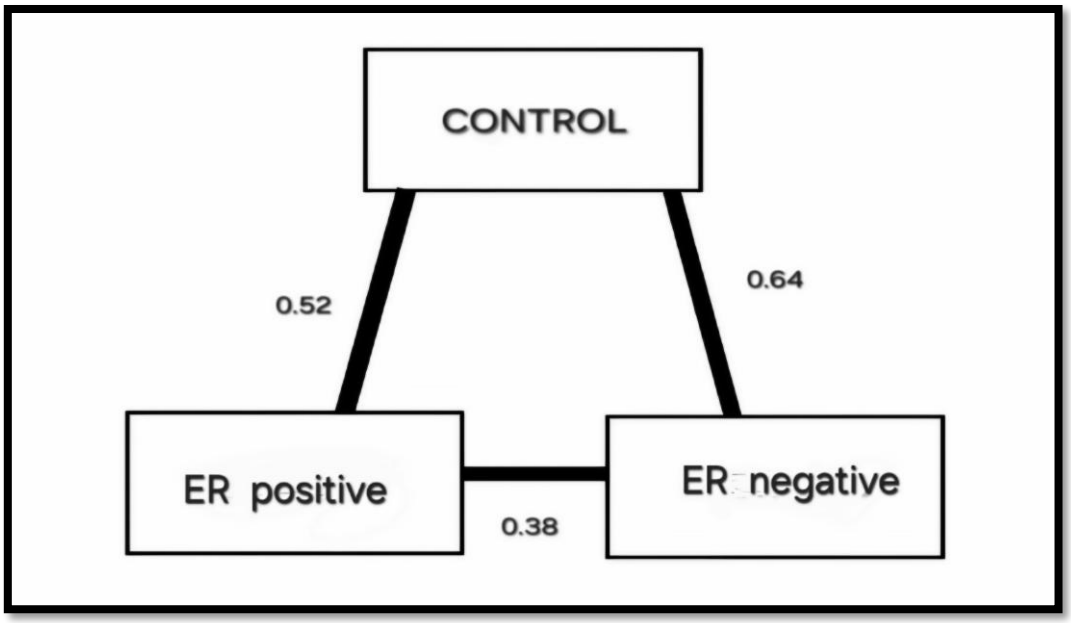

Figure (3) is a diagram illustrating the relationships between the three CCL2 chemokine groups

Figure 4 presents relationships between the three groups in respect of CXCL8 levels and shows a considerable rise in CXCL8 levels for both the ER-positive and ER-negative groups relative to the control group. Moreover, this figure supports results obtained in Table 6, according to which the highest correlation was between the control group and ER-positive groups, whereas the lowest correlation was between the control group and ER-negative groups.

Table (6) Pearson Correlation ® coefficient for CXCL8 level between different groups in terms of estrogen receptor (ER)

The relationship between the groups CXCL8	Correlation coefficient (r)	Statistical interpretation
Control & ER Negative	r = 0.405	weak to moderate correlation
Control and ER positive	r = 0.622	Moderate to strong correlation
ER negative and ER positive	r = 0.537	Average correlation

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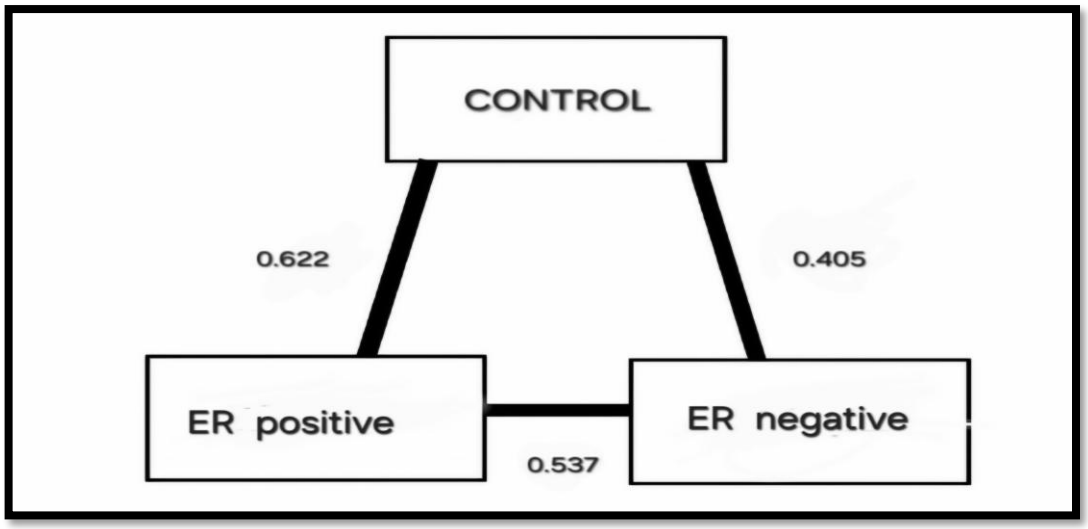

Figure (4) is a diagram illustrating the relationships between the three CXCL8 chemokine groups.

Hormonal factors and especially estrogen hormones together with their receptors are considered to be of utmost importance for the processes of breast cancer development, acting as markers not only for tumor classification but also for regulation of inflammatory signaling pathways within the microenvironment of the tumor tissue. It has been revealed recently that estrogen is capable of influencing gene expression of many cytokines and chemokines by way of activation of certain signaling pathways, such as phosphatidylinositide 3-kinase/protein kinase B and nuclear transcription factor kappa B, thereby creating favorable conditions for tumor growth and development [12] [13]. The current research on the link between estrogen receptors and inflammatory markers of breast cancer shows that there is a considerable rise in the mean values of CCL2 and CXCL8 among breast cancer patients. This is explained by the important role played by these chemokines in regulating the microenvironment of the tumor tissue. CCL2 is one of these chemokines, whose function is related to immune cell mobilization, mainly of macrophages, to the site of the tumor, resulting in modification of the immune microenvironment to one favorable to tumor growth. Recent studies suggest that CCL2 can be found in larger amounts in breast cancer tissues than in normal tissues. Besides, CCL2 promotes tumor perfusion, cancer cell survival, and metastasis formation [11].

CXCL8 is a chemokine of the CXC family and plays a vital role in promoting cell migration, tumor perfusion, and metastasis via its receptors CXCR1 and CXCR2

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through enhanced signaling related to the epithelial-to-mesenchymal transition [14] Recent evidence indicates that upregulation of CXCL8 has been linked to advanced stages of breast cancer and poor outcomes for this condition [14] Recent evidence shows that chemokines of this type are not just indicators of inflammation but are involved in the regulation of intratumoral immunity.

In addition, the role of CXCR2 receptors in the heterogeneity of effects of CXCL8 on tumor proliferation, perfusion, and inflammation can suggest that regulation of CXCL8 expression is determined not only by the estrogen receptor status of the tumor but also by the interaction of this protein with other signaling pathways in the tumor [15] This conclusion is based on the fact that the currently accepted immunological theory considers that elevation of the level of CCL2 and CXCL8 chemokines reflects the presence of inflammation within the tumor tissue. Chemokines can attract and activate immune cells that will migrate to the tumor tissue, where some of them, such as tumor-associated macrophages, will change the function from destructive for the tumor to a supporting one. Therefore, these studies show that imbalance of chemokine receptors and their proteins play a key role in modulation of local immunity and the subsequent development of the tumor tissue [16] [17]

Some studies indicate variability in the correlation between CXCL8 and ER-status of breast cancer patients. According to one of the studies, a higher level of CXCL8 was found in more malignant breast cancer types, such as triple negative breast cancer, in comparison with estrogen-positive tumors. These results may be due to different factors affecting the interaction of chemokine CXCL8 with receptors, such as the status of other growth receptors, clinical features of the tumor, or the analysis method used [18] Based on these findings, we can draw a conclusion about the increased inflammatory activity of the tumor environment due to elevated levels of CCL2 and CXCL8 proteins in breast cancer. In addition, the impact of the estrogen receptor status should also be considered, which plays a key role in this inflammatory activity.

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The Effect of Progesterone Receptor (PR) Status on CCL2 and CXCL8 Chemokines and Their Relationship to the Inflammatory Response in Breast Cancer Patients

Table (7) shows that the concentrations of the chemokines CCL2 and CXCL8 were elevated in breast cancer patients compared to the control group when classified according to progesterone receptor (PR) status. The mean CCL2 in the control group was 0.1961 ± 0.4572, while it increased to 0.6822 ± 0.1447 in the PR-negative group and to 0.5949 ± 0.0878 in the PR-positive group. These differences were statistically significant (p-value = 0.123). Similarly, the mean CXCL8 in the control group was 0.2728 ± 0.9019, while it increased to 1.0453 ± 0.1586 in the PR-negative group and to 1.0938 ± 0.1443 in the PR-positive group. The differences were statistically significant at a p-value of 0.193.

Table (7) Comparison of mean CCL2 and CXCL8 concentrations between the control group and breast cancer patients as indicated by progesterone receptors

Variables	Parameters	Mean ± SD	p-value	LDS value
CCL2	**Control**	0.1961 ± 0.4572	0.0022	0.123
	negativ PR	0.1447 ± 0.6822		
	Positiv PR	0.0878 ± 0.5949		
CXCL8	**Control**	0.9019 ± 0.2728	00.457	0.193
	negative PR	1.0453 ± 0.1586		
	Positive PR	1.0938 ± 0.1443		

Table (8) of the Pearson correlation coefficient for CCL2 showed a weak negative correlation between control and negative PR (r = -0.21) and a weak negative correlation between control and positive PR (r = -0.32). A moderate positive correlation was observed between the positive and negative PR groups (r = +0.64). Figure (5) supported these results by showing a relative convergence between the positive and negative PR groups compared to their difference from the control group. For CXCL8,

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Table (8) Pearson Correlation (r) coefficient for CCL2 levels between different groups in terms of progesterone receptors

The relationship between the groups CCL2	Correlation coefficient (r)	Statistical interpretation
Control & ER Negative	r =- 0.21	Weak negative (reverse) correlation
Control and ER positive	r =-0.32	Weak (negative) correlation
ER negative and ER positive	r = 0.64	Moderate positive correlation (positive)

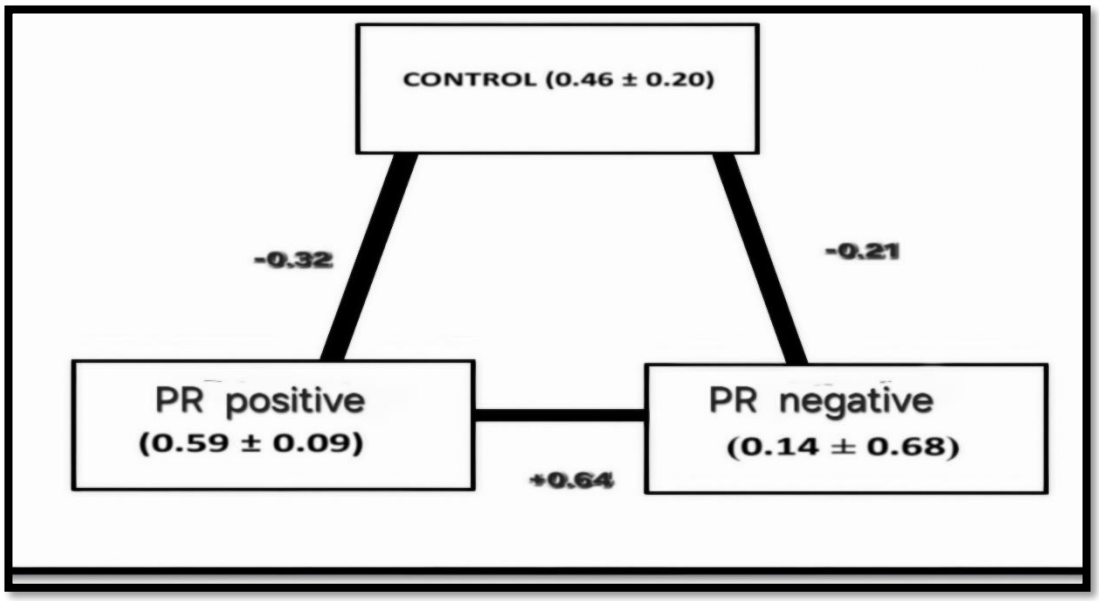

Figure (5) is a diagram illustrating the relationships between the three CCL2 chemokine groups.

Table (9) of the Pearson correlation coefficient showed a weak negative correlation between control and negative PR (r = -0.24) and a weak negative correlation between control and positive PR (r = -0.31). A very strong positive correlation was recorded between the positive and negative PR groups (r = +0.81). Figure (6) clearly supported these results by showing a strong convergence between the two diseased groups. Thus, a distinct difference between the control group implies that increased expression of the proteins CCL2 and CXCL8 is associated more closely with tumor characteristics than PR status changes.

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Table (9) Pearson Correlation (r) coefficient for CXCL8 levels between different groups in terms of progesterone receptors

The relationship between the groups CXCL8	Correlation coefficient (r)	Statistical interpretation
Control & ER Negative	r = -0.24	Inverse weak correlation
Control and ER positive	r = -0.31	Weak inverse correlation
ER negative and ER positive	r = +0.81	Very strong correlation, direct correlation.

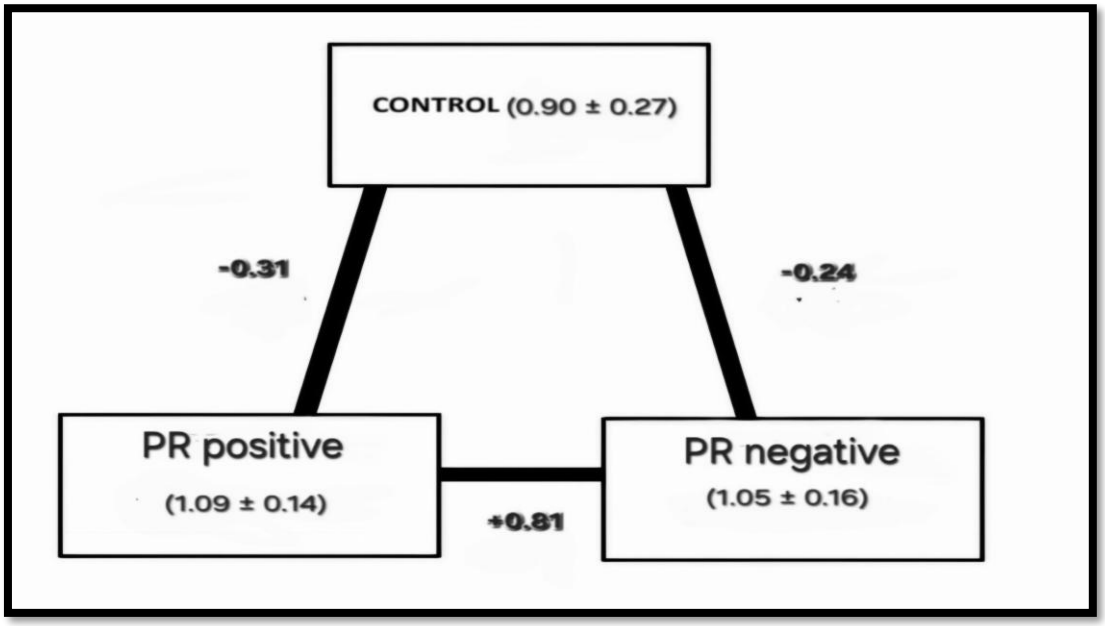

Figure (6) is a diagram illustrating the relationships between the three CXCL8 chemokine groups.

Recent literature data showed that the progesterone receptor (PR) is an essential biomarker for breast cancer due to its relation to the differentiation of tumors and effectiveness of hormonal therapy. Loss or reduction in the PR content can also serve as a sign of increased aggressiveness of tumor development in certain molecular profiles. According to the research conducted by [19], PR status correlates with the clinical prognosis of the patient, and patients who are characterized as PR-negative demonstrate lower survival rates. The findings of the current paper revealed a significant increase in the concentration of CCL2 and CXCL8 chemokines in the blood serum of patients suffering from breast cancer compared to the control group after stratification by PR status. This finding is quite expected based on previous studies concerning the impact of inflammatory chemokines on tumor development. The CCL2

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chemokine recruits' macrophages into tumor tissue, while CXCL8 contributes to angiogenesis, metastasis, and invasion of cancer cells. According to [20], clinical benefits of the progesterone receptor (PR) extend far beyond prediction of hormonal therapy.

These results indicate intratumoral biological processes that include inflammation and cellular signaling pathways. As the results of the current study demonstrate, the high CCL2 and CXCL8 levels might reflect the activity of the inflammatory response inside the tumor. Furthermore, the progesterone receptor status may correlate with the intensity of the inflammatory response inside the tumor microenvironment. The integration of the analysis of progesterone receptors with inflammatory markers such as CCL2 and CXCL8 may help better understand the behavior and development of tumors. Specifically, the role of various hormones might be more comprehensively explained. The chemokines discussed here are related to the enhancement of the tumor microenvironment via recruiting immune cells and chronic inflammation, which contributes to tumor development and aggression. Recent research has confirmed the involvement of the CXCL8-CXCR1/R2 axis in the characteristics and behavior of breast cancer tumors [21]. This finding confirms the current knowledge that the progesterone receptor PR acts not only as an independent hormonal marker but also plays a part in a complicated intratumoral signaling network with estrogen receptor interference and molecular heterogeneity. Moreover, the tumor represents a multifactorial disease due to the relationship between hormonal and immune signals in the tumor microenvironment. [22]

The relationship between CCL2 and CXCL8 levels and the use of therapies (chemotherapy, biological therapy, radiotherapy) in breast cancer patients

The results are presented in Table (10), A significant increase ($p \leq 0.001$) was noted in the amount of CCL2 and CXCL8 in the treatment groups compared to the control group. There was a certain difference in the increase depending on the nature of the treatment. This is connected with the role of chemokines in the development of the inflammatory response induced by the treatment methods and in general biological processes in breast cancer patients. It was found that CCL2 showed a significant increase ($p \leq 0.001$) in the treatment groups compared to the control group. In addition, the maximum level was recorded in the radiotherapy and chemotherapy groups (0.8503

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± 0.0456 and 0.7984 ± 0.0622). This means that radiation therapy stimulated the appearance of the inflammatory response in these patients. These findings are consistent with the literature, in which it is emphasized that CCL2 is elevated in breast cancer. It promotes monocytes recruitment. Moreover, macrophages play a key role in the formation of an inflammatory reaction associated with the progression of breast cancer.

Table (10) Effect of different treatments on CCL2 and CXCL8 levels in women with breast cancer

Variables	Parameters	Mean ± SD	p-value	LDS value
CCL2	Control	0.0994 ± 0.4073	0.0001	0.085
	Biological therapy	0.0522 ± 0.5713		
	Chemotherapy	0.0622 ± 0.7984		
	radiation therapy	0.0456 ± 0.8503		
CXCL8.	Control	0.1526 ± 0.8160	0.001	0.115
	Biological therapy	0.0666 ± 0.9648		
	Chemotherapy	0.1323 ± 1.2116		
	Radiation therapy	0.1446 ± 1.1204		

CXCL8 (IL-8) was found to have a similar pattern of action. The minimum level of this chemokine was recorded in the control group (0.8160 ± 0.1526). Meanwhile, an increase was found in the remaining groups with a maximum level in the chemotherapy group (1.2116 ± 0.1323). Statistically significant results ($p \leq 0.001$) were noted. CXCL8 is characterized by its ability to regulate processes of inflammation, cellular stimulation, and angiogenesis, all of which occur in breast cancer [14]. The results of our research are consistent with those obtained from the Pathway project [25]. The authors demonstrated the importance of the CXCL8-CXCR1/2 axis in breast cancer since it plays an important role in metastasis and structural changes in the cell, and even activates cancer stem cells. Such processes can become the cause of treatment resistance and breast cancer recurrence.

Tables 11 and 12 present the results of Pearson correlation analysis carried out in this work to evaluate the correlation between CCL2 and CXCL8 in various treatment groups. They showed high correlations in the study, mainly between chemotherapy and

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radiotherapy. This indicated the possibility of their synergistic effect on the induction of inflammation, which could occur due to the joint impact of treatment on breast cancer.

Table (11) Analysis of correlation coefficients (Pearson correlation) CCL2

The relationship between CCL2 groups	Correlation coefficient (r)	Statistical interpretation
Control and administering chemotherapy	r =0.74	Strong positive correlation
Control and taking biological treatment	r =0.68	Moderate to strong positive correlation
Control and receiving radiation therapy	r = 0.71	strong positive correlation
He took chemotherapy with a biological agent.	r = 0.82	Very strong positive correlation
He received chemotherapy and radiation therapy.	r =0.88	Very high positive correlation
He received biological and radiation therapy.	r = 0.85	Very strong positive correlation

Table (12) Analysis of correlation coefficients between groups (Pearson Correlation) CXCL8

The relationship between CXCL8 groups	Correlation coefficient (r)	Statistical interpretation
Control and administering chemotherapy	r =0.74	strong positive correlation
Control and taking biological treatment	r =0.84	Very strong positive correlation
Control and receiving radiation therapy	r = 0.76	Strong positive correlation
He took chemotherapy with a biological agent.	r =0.79	strong positive correlation
He received chemotherapy and radiation therapy.	r = 0.88	Very high positive correlation
He received biological and radiation therapy.	r = 0.81	Very strong positive correlation

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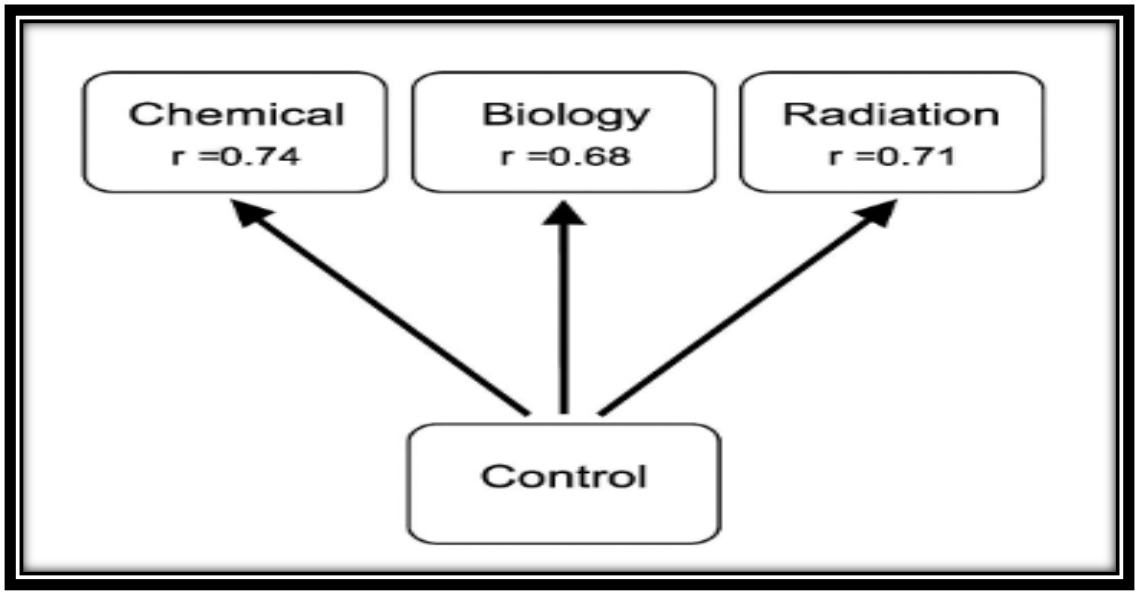

Figure (7) Pearson correlation analysis chart (CCL)

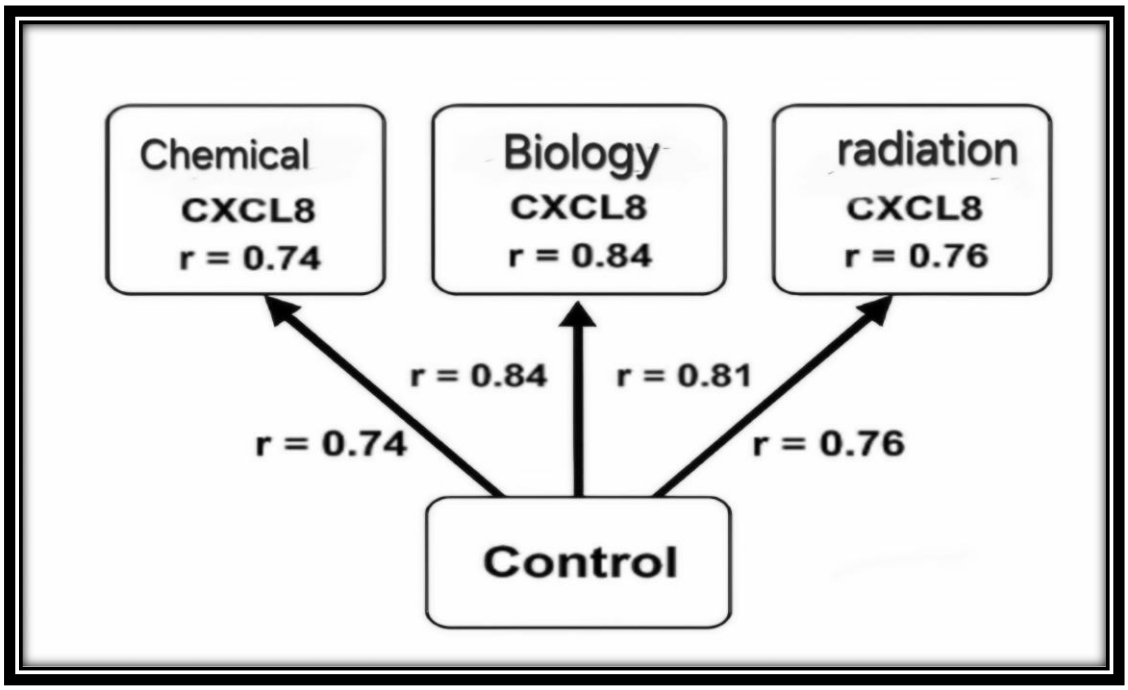

Figure (8) Pearson correlation analysis chart CXCL8

This result confirms the findings of earlier research demonstrating the functioning of CCL2 and CXCL8 in the inflammatory network [26]. As seen from the obtained data of this research, the levels of CCL2 and CXCL8 showed a pronounced correlation at a range from 0.68 to 0.88 depending on the comparison groups. The highest level of

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correlation was registered among patients treated with chemotherapy and radiotherapy (for CCL2 – r = 0.88 and for CXCL8 – r = 0.88), which can be explained by the fact that both methods of treatment can cause significant cellular damage and induce oxidative stress, thus activating inflammatory mechanisms involved in chemokine secretion including NF-κB and STAT3 mechanisms. Previous research indicated that such activation was related to increased production of both types of chemokine from cancerous and peritumor cells, which could explain the observed correlation between CCL2 and CXCL8 levels in this group [17]. As it is seen from Tables 11 and 12, Figures 7 and 8, the strong correlation between biological therapy and chemotherapy/radiotherapy (r = 0.79-0.85) suggests a possibility of overlap of the immune response associated with biological therapy and the inflammatory response induced as a result of chemotherapy and radiotherapy, which might be associated with the role played by CCL2 and CXCL8 in the formation of such response by attracting inflammatory cells or altering the tumor microenvironment. In the case of breast cancer, such concurrent increase in CCL2 and CXCL8 concentrations was associated with aggressive tumors and facilitation of cancer cell migration and angiogenesis, which supports the idea that the high correlation between the two markers might be caused by the enhanced inflammatory response associated with certain treatment methods [27][28].

Conclusion

In this study, it was revealed that two chemokine receptors, CCL2, and CXCL8, play an important part in breast cancer development. The expression levels of CXCL8 were found to be high among patients, suggesting that it is highly associated with inflammation and tumor progression. On the other hand, CCL2 expression levels are varied based on different conditions. Different treatment methods, specifically chemotherapeutic and radiotherapeutic treatment methods, showed significant levels of these two markers, emphasizing the importance of inflammation in such treatment methods. In general, CXCL8 might be used as an inflammatory biomarker for breast cancer because it is constant in all conditions.

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