RESEARCH ARTICLE

# Relation between baseline CXCR1 expression and neoadjuvant chemotherapy response in breast cancer patients

Sara Adel Amin[1], Emad Abd El Mongy Sadaka[2], Khalid Ahmed Ismail[3], Ashraf Fathy Barakat[4], Ahmed Abdel Aziem Menaisy[5], Amr Abd Elmoneim Mahmoud[6]

**Abstract**

**Objective:** To examine the C-X-C Motif Chemokine Receptor 1 expression in breast cancer tissues prior to neo-adjuvant chemotherapy, and its relationship to neo-adjuvant chemotherapy effectiveness and other prognostic variables.

**Method:** The prospective study was conducted at Kafrelsheikh University Hospital, Egypt, from November 2018 to March 2021, and comprised patients with recent histopathologically proven breast cancer cases eligible for chemotherapy. Paraffin blocks of tumour specimens were stained by immunohistochemical stain using concentrating rabbit anti-human C-X-C Motif Chemokine Receptor 1 polyclonal antibody kits. C-X-C Motif Chemokine Receptor 1 expression was classified into low and high categories. Patients were followed for 2 years for treatment response, disease recurrence and mortality. Data was analysed using SPSS 25.

**Results:** Of the 100 females with mean age 50.2±12.1 years, 52(52%) had their left side affected, while 48(48%) had their right side affected. There were 52(52%) cases with mean age 49.2±12.9 years having high C-X-C Motif Chemokine Receptor 1 expresssion, while 48(48%) with mean age 51.4±11.2 years had low expression. There was a significant association between high expression and advanced tumour grade, advanced tumour stage, higher frequency of triple negative breast cancer and higher frequency of Ki-67-positive cancers ($p<0.05$). Patients with high C-X-C Motif Chemokine Receptor 1 expression had significantly lower frequency of complete pathological response when compared with patients with low expression ($p<0.001$). Patients with high expression had higher frequency of recurrence, shorter disease-free survival, higher mortality and shorter overall survival, but the difference was not significant ($p>0.05$). Multivariate logistic regression analysis identified triple negative hormonal status ($p=0.031$) and high baseline C-X-C Motif Chemokine Receptor 1 expression ($p<0.001$) as significant predictors of complete pathological response.

**Conclusions:** There was found to be a link between baseline C-X-C Motif Chemokine Receptor 1 expression in breast cancer tissues and pathological response to neoadjuvant therapy in breast cancer patients.

**Keywords:** Neoadjuvant therapy, Prognosis, Ki67 proliferation marker, Paraffin, Immunohistochemistry, Breast neoplasms, Chemokines. **DOI: 10.47391/JPMA.EGY-S4-8**

## Introduction

Breast cancer (BC) is the most common malignancy in women in the United States, and the second leading cause of cancer death after lung cancer.[1] Similarly, BC is the most common cancer among Egyptian women, accounting for 16.4% of all malignancies.[2] The development of BC is a highly complex process that involves several oncogenes, tumour suppressor genes, and transcription factors. However, the exact process is still unknown.[3]

Chemokines' role in tumour formation has gained a lot of attention in recent years. C-X-C chemokines and their receptors can affect some types of cancers. These released chemicals may have anti-cancer properties by affecting the tumour cell chemotaxis. In addition, they stimulate tumour growth and spread by increasing angiogenesis and extracellular matrix digestion.[4,5] In vitro usage of antibodies specific to C-X-C Motif Chemokine Receptor 1 (CXCR1) and CXCR2 has been shown to decrease melanoma tumour growth.[6]

There are at least 20 different types of chemokine receptors known to date.[7] Prostate cancer, bladder cancer, stomach cancer, colon cancer, endometrial cancer and melanoma show high levels of CXCR1 expression.[8] CXCR1 is also thought to have a significant role in the development and management of BCs, and could be used as an indication of neoadjuvant chemotherapy (NAC) effectiveness.[8-10]

The current study was planned to examine CXCR1 expression in BC tissues prior to NAC, and its link to NAC effectiveness and other prognostic factors.

[1,2,6] Department of Clinical Oncology and Nuclear Medicine, Kafrelsheikh University, Egypt.

[3]Department of General and Paediatric Surgery, Kafrelsheikh University, Egypt.

[4]Department of Clinical Oncology and Nuclear Medicine, Tanta University, Egypt.

[5] Department of Pathology, Tanta University, Egypt.

**Corrospondience:** Sara Adel Amin email: sara.islam84@gmail.com

## Patients and Methods

The prospective study was conducted at Kafrelsheikh University Hospital, Egypt, from November 2018 to March 2021. After approval from the institutional ethics review committee, the sample was conveniently raised from among newly diagnosed histopathologically-proven breast cancer women during the period November 2018 to March 2021 and meeting the inclusion criteria of being diagnosed with recent histopathologically proven breast cancer and eligible for chemotherapy. Patients with recurring or metastatic disease, bilateral BCs, or non-epithelial origin BCs, such as phyllodes tumour, sarcoma or lymphoma, were excluded.

Data was collected after taking written informed consent from the subjects. All patients underwent complete clinical examination and routine laboratory investigations, like complete blood count (CBC), liver function test (LFT), and tests related to the renal functions, and radiological assessment, like mammography, breast ultrasonic examination, chest X-ray (CXR) and/ or computed tomography (CT), pelvi-abdominal ultrasound and/ or CT, bone scan and echocardiography.

Paraffin blocks of pre-operative tumour specimens were subjected to immunohistochemical (IHC) examination for hormonal receptors status, including oestrogen (ER), progesterone (PR), human epidermal growth factor receptor 2 (HER2) and Ki67. Blocks were also re-sectioned at 4 microns and stained by IHC stain using concentrated rabbit anti-human CXCR1 polyclonal antibody kits (AB Clonal, United States) to assess the expression of CXCR1. CXCR1 expression was classified into low and high categories. Five vision fields were taken from the sections. The positive cells percentage and staining intensity in each vision were used to calculate the score. The score of positive cells percentage was allocated based on the proportion of positive cells in view of total cell population as follows: <10%=0 points, 10%~25% = 1 point, 26% ~ 50% = 2 points, 50%~75%=3 points and >75%=4 points. The staining intensity score was assigned by dyed colour as follows: positive cells without colouring noted=0 points, dyed pale yellow noted=1-point, dyed tan noted=2 points, and dyed brown noted=3 points. [8]

All patients were treated by NAC regimen comprising adriamycin 60mg/m$^2$ and cyclophosphamide 600mg/m$^2$ intravenously (IV) on day 1 and every 21 days for 4 cycles based on the assessment of clinical status. The patients underwent surgical treatment either breast conservative surgery (BCS) or modified radical mastectomy (MRM) with axillary dissection. In addition, they received adjuvant protocol taxol 80mg/m$^2$ weekly for 12 weeks. Moreover, patients received post-operative radiation therapy. Other treatment options included target therapy and hormonal therapy according to hormonal status and HER2 expression.

Haematoxylin and Eosin (H&E) was used to stain the surgical samples, and a microscope (Olympus) was used to view the organisational structure. Tumour size, lymph node (LN) status, histological grade and pathological type were evaluated along the pathological process. Additionally, the surgical specimens were graded using the Miller-Payne classification. Grades 1 and 2 indicated a mild response to NAC, grade 3 a moderate response and grades 4 and 5 a complete pathological response. [11]

The patients were followed up for 2 years; every 3 months in the first year, and every 6 months in the second year. The follow-up included clinical examination and laboratory and radiological assessment. Patients were followed for disease recurrence and mortality.

Data was analysed using SPSS 25. Data was expressed as frequencies and percentages, or as mean and standard deviation, as appropriate. The t test was used to evaluate numerical data, and chi-square test was used to analyse categorical data. To find determinants of therapy response, logistic regression analysis was used. In order to assess disease-free survival (DFS) and overall survival (OS) between patients with low and high CXCR1 expressions, Kaplan-Meier survival analysis with log-rank comparison was used. P<0.05 was deemed statistically significant.

## Results

Of the 100 females with mean age 50.2±12.1 years, 52(52%) had their left side affected, while 48(48%) had their right side affected. There were 52(52%) cases with mean age 49.2±12.9 years having high CXCR1expresssion, while 48(48%) with mean age 51.4±11.2 years had low expression. There was a significant association between high expression and advanced tumour grade, advanced tumour stage, higher frequency of triple negative breast cancer (TNBC) and higher frequency of Ki-67-positive cancers ($p$<0.05). Patients with high CXCR1expression had significantly lower frequency of complete pathological response when compared with patients with low expression ($p$<0.001). Patients with high expression had higher frequency of recurrence, shorter OS (Figure 1), higher mortality and shorter DFS (Figure 2), but the difference was not significant ($p$>0.05) (Table 1).

Multivariate logistic regression analysis identified triple negative hormonal status (p=0.031) and high baseline CXCR1 expression ($p$<0.001) as significant predictors of

**Table-1:** Clinical, pathological and outcome parameters (n=100).

| | All patients N=100 | High CXCR1 n=52 | Low CXCR1 n=48 | *p-value* |
|---|---|---|---|---|
| **Mean Age** (years) | 50.2 ± 12.1 | 49.2 ± 12.9 | 51.4 ± 11.2 | 0.35 |
| **Affected side** n (%) | | | | |
| Right | 48 (48.0) | 28 (53.9) | 20 (41.7) | 0.24 |
| Left | 52 (52.0) | 24 (46.1) | 28 (58.3) | |
| **Tumour pathology** n (%) | | | | |
| Ductal carcinoma | 70 (70.0) | 33 (63.5) | 37 (77.1) | 0.47 |
| Lobular carcinoma | 12 (12.0) | 8 (15.4) | 4 (8.3) | |
| Mucinous carcinoma | 9 (9.0) | 5 (9.6) | 4 (8.3) | |
| Papillary carcinoma | 9 (9.0) | 6 (11.5) | 3 (6.3) | |
| **Tumour grade** n (%) | | | | |
| Moderate differentiated | 88 (88.0) | 40 (76.9) | 48 (100.0) | <0.001 |
| Poor differentiated | 12 (12.0) | 12 (23.1) | - | |
| **Tumour stage** n (%) | | | | |
| I | 16 (16.0) | - | 16 (33.3) | <0.001 |
| II | 48 (48.0) | 20 (38.5) | 28 (58.3) | |
| III | 36 (36.0) | 32 (61.5) | 4 (8.3) | |
| **Hormonal receptors status** n (%) | | | | |
| ER+ve | 56 (56.0) | 16 (30.8) | 40 (83.3) | <0.001 |
| PR+ve | 52 (52.0) | 12 (23.1) | 40 (83.3) | <0.001 |
| Her2 +3 | 32 (32.0) | 24 (46.1) | 8 (16.7) | <0.001 |
| Triple negative | 28 (28.0) | 20 (38.5) | 8 (16.7) | 0.025 |
| **Ki67 high index** n (%) | 68 (68.0) | 44 (84.6) | 24 (50.0) | <0.001 |
| **Treatment response** n (%) | | | | |
| Mild | 28 (28.0) | 20 (38.5) | 8 (16.7) | <0.001 |
| Moderate | 32 (32.0) | 28 (53.9) | 4 (8.3) | |
| Marked | 40 (40.0) | 4 (7.7) | 36 (75.0) | |
| **Recurrence** n (%) | 11 (11.0) | 8 (15.4) | 3 (6.3) | 0.13 |
| **DFS** (months) mean (95% CI) | 22.9 (22.2-23.5) | 22.1 (20.9-23.3) | 23.7 (23.4-24.0) | 0.18 |
| **Mortality** n (%) | 7 (7.0 %) | 5 (9.6) | 2 (4.2) | 0.25 |
| **OS** (months) mean (95% CI) | 29.2 (28.6-29.9) | 23.5 (23.0-23.9) | 29.7 (28.8-30.6) | 0.087 |

CXCR1: C-X-C Motif Chemokine Receptor 1, ER: Oestrogen, PR: Progesterone, DFS: Disease-free survival, OS: Overall survival, CI: Confidence interval.

**Table-2:** Predictors of marked treatment response.

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | p | OR | 95% CI | P |
| Tumour grade | 0.72 | 0.2-2.58 | 0.62 | - | - | - |
| Triple negative | 6.0 | 1.89-19.04 | 0.002 | 5.92 | 1.17-29.86 | 0.031 |
| Ki67 | 4.0 | 1.65-9.7 | 0.002 | 0.84 | 0.21-3.32 | 0.81 |
| CXCR1 | 0.028 | 0.008-0.093 | <0.001 | 0.027 | 0.007-0.11 | <0.001 |

OR: Odds ratio, CI: Confidence interval, CXCR1: C-X-C Motif Chemokine Receptor 1.

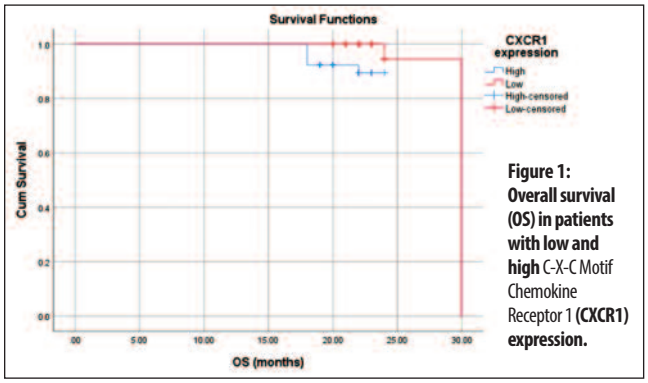


**Figure 1: Overall survival (OS) in patients with low and high** C-X-C Motif Chemokine Receptor 1 **(CXCR1) expression.**

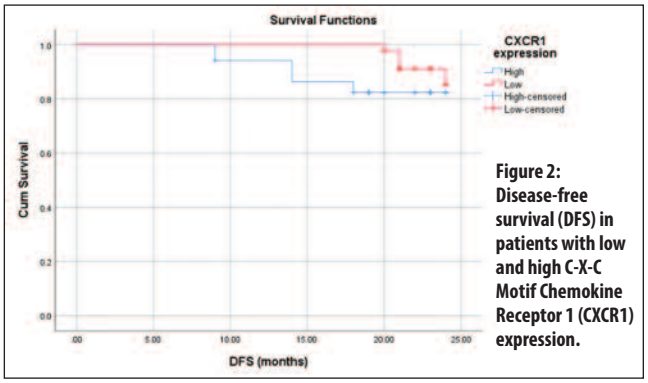


**Figure 2: Disease-free survival (DFS) in patients with low and high C-X-C Motif Chemokine Receptor 1 (CXCR1) expression.**

complete pathological response (Table 2).

## Discussion

In the current study, high CXCR1 expression was found to be significantly correlated with advanced tumour stage, advanced tumour grade, more TNBCs, and more Ki67-positive malignancies. The findings are consistent with an earlier study.[8] Additionally, Chen et al.[12] noted that C-X-C ligand 8 (CXCL8; (a CXCR1 ligand) levels were higher in ER-negative, PR-negative and HER2-positive cancers. Additionally, they reported that TNBC patients had considerably higher levels of CXCL8 messenger ribonucleic acid (mRNA) expression. These findings imply that the level of BC malignancy is correlated with CXCR1.

In the current study, patients with high CXCR1 expression had significantly lower frequency of complete pathological response compared to patients with low expression (7.7% versus 75%, $p<0.001$). This is in agreement with literature[8].

The current findings may have important therapeutic ramifications as the therapeutic response may be enhanced further if CXCR1 expression is suppressed using CXCR1 blockers. Reparixin, a potent CXCR1 inhibitor, has been found by Brandolini et al.[10] to be helpful in lowering tumour development and recurrence.

## Conclusion

The findings suggest an association between baseline CXCR1 expression in BC tissues and pathological response to NAC.

**Limitation:** The sample size for the study was not calculated which can have an adverse effect on the power of the study.

**Disclaimer:** None.

**Conflict of Interest:** None.

**Source of Funding:** None.

## References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30. doi: 10.3322/caac.21590.
2. World Health Organization (WHO), International Agency for Research on Cancer (IARC). The Global Cancer Observatory: Egypt. [Online] 2020 [Cited 2021 March 05]. Available from URL: https://gco.iarc.fr/today/data/factsheets/populations/818-egypt-fact-sheets.pdf.
3. Ramya Sree PR, Thoppil JE. An overview on breast cancer genetics and recent innovations: Literature survey. Breast Dis 2021;40:143-54. doi: 10.3233/BD-201040.
4. Helbig G, Christopherson KW, Bhat-Nakshatri P, Kumar S, Kishimoto H, Miller KD, et al. NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. J Biol Chem 2003;278:21631-8. doi: 10.1074/jbc.M300609200.
5. Palacios-Arreola MI, Nava-Castro KE, Castro JI, García-Zepeda E, Carrero JC, Morales-Montor J. The role of chemokines in breast cancer pathology and its possible use as therapeutic targets. J Immunol Res 2014;2014:849720. doi: 10.1155/2014/849720.
6. Sapoznik S, Ortenberg R, Galore-Haskel G, Kozlovski S, Levy D, Avivi C, et al. CXCR1 as a novel target for directing reactive T cells toward melanoma: implications for adoptive cell transfer immunotherapy. Cancer Immunol Immunother 2012;61:1833-47. doi: 10.1007/s00262-012-1245-1.
7. Rollins BJ. Inflammatory chemokines in cancer growth and progression. Eur J Cancer 2006;42:760-7. doi: 10.1016/j.ejca.2006.01.002.
8. Xue MQ, Liu J, Sang JF, Su L, Yao YZ. Expression characteristic of CXCR1 in different breast tissues and the relevance between its expression and efficacy of neo-adjuvant chemotherapy in breast cancer. Oncotarget 2017;8:48930-7. doi: 10.18632/oncotarget.16893.
9. Lohri C, Schaltegger CS, VAN DEN Broek M, Wenger RH, Ruegg C, Fink D, et al. Neutrophil expression of ICAM1, CXCR1, and VEGFR1 in patients with breast cancer before and after adjuvant chemotherapy. Anticancer Res 2014;34:4693-9.
10. Brandolini L, Cristiano L, Fidoamore A, De Pizzol M, Di Giacomo E, Florio TM, et al. Targeting CXCR1 on breast cancer stem cells: signaling pathways and clinical application modelling. Oncotarget 2015;6:43375-94. doi: 10.18632/oncotarget.6234.
11. Ogston KN, Miller ID, Payne S, Hutcheon AW, Sarkar TK, Smith I, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. Breast 2003;12:320-7. doi: 10.1016/s0960-9776(03)00106-1.
12. Chen E, Qin X, Peng K, Xu X, Li W, Cheng X, et al. Identification of Potential Therapeutic Targets Among CXC Chemokines in Breast Tumor Microenvironment Using Integrative Bioinformatics Analysis. Cell Physiol Biochem 2018;45:1731-46. doi: 10.1159/000487782.