RESEARCH ARTICLE

Clinical significance of baseline and post-treatment CXCR1 expression in women with breast cancer having received neoadjuvant chemo therapy

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Abstract

Objective: To examine the chemokine receptor type 1 expression in breast cancer tissues before and after neoadjuvant chemotherapy, and its relationship with pathological response to neoadjuvant chemotherapy and other clinical variables.

Methods: The prospective study was conducted at Kafrelsheikh University Hospital, Egypt, from November 2018 to March 2021, and comprised female patients with new histopathologically proven breast cancer eligible for chemotherapy. Paraffin blocks of tumour specimens were stained immunohistochemically using concentrated rabbit anti-human chemokine receptor type 1 polyclonal antibody kits. The patients were followed up for treatment response, disease recurrence and mortality. Data was analysed using SPSS 25.

Results: Of the 100 patients with mean age 50.2±12.1 years, 40(40%) in group A with mean age 55.1±9.3 showed marked response and 60(60%) in group B with mean age 47.0±12.7 years showed mild/moderate response (p<0.001). Group A patients had significantly lower baseline and post-treatment chemokine receptor type 1 expression compared to group B patients (p<0.05). The change in chemokine receptor type 1 expression was not significantly different (p>0.05). Patients with tumour grade 3 had significantly higher baseline chemokine receptor type 1 expression compared to patients with tumour grade 2. Tumour stage and post-treatment chemokine receptor type 1 expression were also significantly interlinked (p<0.05). Multivariate regression analysis identified patients' age, baseline chemokine receptor type 1 and post-treatment chemokine receptor type 1 expressions as predictors of treatment response.

Conclusions: There was found to be an association between baseline and post-treatment chemokine receptor type 1 expression in breast cancer tissues and pathological response to neoadjuvant chemo therapy in such patients.

Keywords: Neoadjuvant therapy, Paraffin, Breast neoplasms, Receptors, Chemokine.

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Introduction

Breast cancer (BC) incidence continues to rise in low- and middle-income countries (LMICs), including Egypt.[1] The gold standard care in BC management is neoadjuvant therapy, which may include cytotoxic or endocrine therapy, before surgery.[2] However, in spite of the standardisation of treatment depending on the genetic and endocrine characteristics of tumours, the treatment response is not the same in every patient, making identification of new treatment targets a clinical priority in such patients.[3]

The significance of chemokines and chemokine receptors in cancer development has received significant attention in recent years. They were found to be implicated in the development, angiogenesis, metastasis, and treatment resistance of BC. Chemokine and chemokine receptor regulation may become a novel technique for BC therapy.[4] Chemokines play a variety of roles when released by diverse immune cells in the tumour microenvironment of BC, which can be broadly classified as immunosuppressive or immunostimulatory.[5] C-X-C chemokine ligand 8 (CXCL8) and its receptors C-X-C chemokine receptor type 1 (CXCR1) and CXCR2 are released by cancer cells. Patients of prostate cancer, bladder cancer, stomach cancer, colon cancer, endometrial cancer and melanoma have all been found to have high levels of CXCR1.[6]

CXCR1 affects cell adhesion, proliferation, migration and metastasis, which inhibit the growth of tumours and the activation and trafficking of inflammatory mediators.[7] Neoadjuvant chemotherapy (NAC) response could also be successfully predicted by CXCR1.[8]

The current study was planned to investigate CXCR1 expression in BC tissues before and after NAC, as well as its relationship with NAC efficacy and other clinical variables.

Patients and Methods

The prospective study was conducted at Kafrelsheikh

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University Hospital, Egypt, from November 2018 to March 2021. After approval from the institutional ethics review committee, the sample was raised from among adult female patients with new histopathologically proven BC eligible for chemotherapy. Patients were excluded if they had recurrent or metastatic disease, bilateral BCs or BC of non-epithelial origin, such as phyllodes tumour, sarcoma or lymphoma.

After written informed consent, all patients underwent complete clinical examination and routine laboratory investigations, like complete blood count (CBC), liver functions test (LFT) and renal functions test. Radiological assessment included 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 receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), and protein Ki67. Blocks were also re-sectioned at 4 microns and stained by IHC stain by 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 groups. Five vision fields were taken from the section. The percentage of positive cells 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 = 0 points, dyed pale yellow = 1 point, dyed tan - 2 points, and dyed brown - 3 points. Histopathological assessment was performed before and after NAC.

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 then underwent surgical treatment, either breast conservative surgery (BCS) or modified radical mastectomy (MRM) with axillary dissection. In addition, they received adjuvant chemotherapy 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 the hormonal status and HER2 expression

Haematoxylin and eosin (H&E) stain 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 in which grades 1 and 2 showed a slight response to chemotherapy, grade 3 a moderate response, and grades 4 and 5 a significant response.[8]

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

Data was analysed using SPSS 25. Data was presented as frequencies and percentages or mean and standard deviation, as appropriate. Numerical data was compared using t test or one-way analysis of variance (ANOVA), while categorical data was compared using Chi-square test. Correlations were made using Pearson's correlation coefficient. P<0.05 was considered statistically significant.

Results

Convenient sampling technique was used to select 100 patients for the study. The mean age of the group was 50.2±12.1 years. There were 40(40%) in group A with mean age 55.1±9.3 years showing a marked response and 60(60%) in group B with mean age 47.0±12.7 years having mild/moderate response (p<0.001). Pathological complete response had significantly higher frequency of patients with stage II and III tumours, and there was a significant association between marked pathological response and positive ER and PR status (p<0.05). Patients with marked response had significantly lower baseline (p<0.001) and post-treatment (p<0.001) CXCR1 expression compared to patients with mild/moderate response. No significant differences were found between the groups regarding the change in CXCR1 expression (p>0.05). Patients with marked pathological response had significantly lower local recurrence and/or distant metastasis (mets) (p=0.027) and mortality (Table 1).

Patients with tumour grade 3 had significantly higher baseline CXCR1 expression when compared with patients with tumour grade 2 (p<0.001). Patients with tumour stage III had significantly higher baseline, post-treatment, and change of CXCR1 expression when compared with patients with tumour stages I and II (p<0.05). Significantly lower baseline and post-treatment CXCR1 expressions were noted in patients with positive ER and PR, while patients with positive HER2 and Ki67 had significantly higher baseline and post-treatment CXCR1 expression (Table 2).

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

	All patients n=100	Marked Response n=40	Mild/moderate response n=60	*p-value*
Age (years) mean ± SD	50.2 ± 12.1	55.1 ± 9.3	47.0 ± 12.7	<0.001
Affected side n (%)				
Right	48 (48.0)	22 (55.0)	26 (43.3)	0.25
Left	52 (52.0)	18 (45.0)	34 (56.7)	
Tumour pathology n (%)				
Ductal carcinoma	70 (70.0)	24 (60.0)	46 (76.7)	0.31
Lobular carcinoma	12 (12.0)	7 (17.5)	5 (8.3)	
Mucinous carcinoma	9 (9.0)	5 (12.5)	4 (6.7)	
Papillary carcinoma	9 (9.0)	4 (10.0)	5 (8.3)	
Tumour grade n (%)				
2	88 (88.0)	36 (90.0)	52 (86.7)	0.62
3	12 (12.0)	4 (10.0)	8 (13.3)	
Tumour stage n (%)				
I	16 (16.0)	16 (40.0)	-	<0.001
II	48 (48.0)	20 (50.0)	28 (46.7)	
III	36 (36.0)	4 (10.0)	32 (53.3)	
Hormonal receptors status n (%)				
ER				
+ve	56 (56.0)	32 (80.0)	22 (36.7)	
<0.001				
-ve	44 (44.0)	8 (20.0)	38 (63.0)	
PR				
+ve	52 (52.0)	32 (80.0)	20 (33.3)	<0.001
-ve	48 (48.0)	8 (32.0)	40 (66.7)	
Her2				
+ve	32 (32.0)	8 (20.0)	24 (40.0)	0.036
-ve				
Triple negative				
Yes	28 (28.0)	4 (10.0)	24 (40.0)	0.001
No	72 (72.0)	36 (90.0)	36 (60.0)	
Ki67 n (%)				
High	68 (68.0)	20 (50.0)	48 (80.0)	0.002
Low	32 (32.0)	20 (50.0)	12 (20.0)	
CXCR1 expression mean ± SD				
Baseline	5.8 ± 0.7	5.3 ± 0.6	6.1 ± 0.5	<0.001
Post-treatment	3.8 ± 1.0	3.3 ± 0.9	4.2 ± 0.9	<0.001
reduction	1.9 ± 1.0	2.0 ± 1.2	1.9 ± 0.8	0.59
Recurrence/mets n (%)	11 (11.0 %)	1 (2.5)	10 (16.7)	0.027
Mortality n (%)	7 (7.0 %)	-	7 (11.7)	0.025

SD: Standard deviation, ER: Oestrogen receptor, PR: Progesterone receptor, Her2: Human epidermal growth factor receptor 2, CXCR1: Chemokine receptor type 1, Mets: Metastasis.

No significant differences were found between survivors and non-survivors, or between those with recurrence or distant mets and those without regarding baseline and post-treatment CXCR1 expression (Table 3). Multivariate regression analysis identified patients' age, baseline CXCR1 and post-treatment CXCR1 as predictors of marked treatment response (Table 4).

A direct positive association (r=0.4, p=0.001) between pre- and post-treatment CXCR1 expression variations was noted (Figure).

Table-2: CXCR1 expression and pathological findings.

	Baseline CXCR1 expression	Post-treatment CXCR1 expression	CXCR1 expression change
Age			
< 45	5.7 ± 0.7	3.8 ± 0.9	1.9 ± 1.0
≥ 45	5.8 ± 0.7	3.9 ± 1.2	1.9 ± 1.0
p-value	0.37	0.64	0.94
Affected side			
Right	5.7 ± 0.7	3.8 ± 1.0	2.0 ± 1.0
Left	5.8 ± 0.7	3.9 ± 1.1	1.9 ± 1.0
p value	0.58	0.43	0.65

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Table-2: *continued from previous page*

	Baseline CXCR1 expression	Post-treatment CXCR1 expression	CXCR1 expression change
Tumour pathology			
Ductal carcinoma	5.8 ± 0.7	3.9 ± 1.0	1.9 ± 1.0
Lobular carcinoma	5.5 ± 0.6	3.3 ± 1.0	2.2 ± 1.1
Mucinous carcinoma	5.6 ± 0.9	4.2 ± 0.9	1.5 ± 1.1
Papillary carcinoma	5.8 ± 0.6	3.7 ± 1.3	2.1 ± 0.8
p value	0.26	0.35	0.18
Tumour grade			
2	5.7 ± 0.7	3.8 ± 1.1	1.9 ± 1.0
3	6.2 ± 0.2	4.2 ± 0.7	2.0 ± 0.8
p value	< 0.001	0.2	0.77
Tumour stage			
I	4.8 ± 0.3	3.6 ± 1.1	1.2 ± 1.3
II	5.7 ± 0.5	3.5 ± 0.9	2.2 ± 0.8
III	6.3 ± 0.5	4.4 ± 1.0	1.9 ± 0.9
p value	< 0.001	< 0.001	0.001
ER			
+ve	5.4 ± 0.6	3.5 ± 0.8	1.9 ± 1.0
-ve	6.2 ± 0.5	4.2 ± 1.2	1.9 ± 1.0
p value	< 0.001	0.001	0.82
PR			
+ve	5.3 ± 0.5	3.5 ± 0.8	1.9 ± 1.0
-ve	6.2 ± 0.5	4.2 ± 1.1	2.0 ± 1.0
p value	< 0.001	< 0.001	0.49
Her2			
+ve	6.2 ± 0.8	4.4 ± 0.7	1.7 ± 0.6
-ve	5.6 ± 0.5	3.6 ± 1.1	2.0 ± 1.1
p value	0.001	< 0.001	0.097
Triple negative			
No	5.7 ± 0.8	3.8 ± 1.0	1.9 ± 0.9
Yes	5.9 ± 0.3	3.8 ± 1.2	2.0 ± 1.2
p value	0.19	0.97	0.55
Ki67			
High	6.0 ± 0.6	4.0 ± 1.1	2.0 ± 0.9
Low	5.3 ± 0.6	3.5 ± 0.8	1.8 ± 1.1
p value	< 0.001	0.04	0.28

SD: Standard deviation, ER: Oestrogen receptor, PR: Progesterone receptor, Her2: Human epidermal growth factor receptor 2, CXCR1: Chemokine receptor type 1.

Table-3: CXCR1 expression change and association with outcome parameters.

	Baseline CXCR1 expression	Post-treatment CXCR1 expression	CXCR1 expression change
Recurrence/mets			
Yes	5.8 ± 0.3	3.8 ± 0.8	2.1 ± 0.9
No	5.7 ± 0.7	3.8 ± 1.1	1.9 ± 1.0
p value	0.26	0.86	0.55
Mortality			
Yes	5.9 ± 0.3	3.9 ± 0.7	2.0 ± 0.7
No	5.7 ± 0.7	3.8 ± 1.1	1.9 ± 1.0
p value	0.22	0.81	0.85

Mets: Metastasis, CXCR1: Chemokine receptor type 1.

Table-4: Predictors of marked treatment response.

	Univariate analysis			Multivariate analysis		
Age	1.06	1.02-1.1	0.001	1.11	1.02-1.2	0.011
Triple negative	0.17	0.05-0.53	0.002	0.27	0.04-1.9	0.19
Baseline CXCR1	0.06	0.02-0.19	<0.001	0.08	0.025-0.28	<0.001
Posttreatment CXCR1	0.35	0.21-0.57	<0.001	0.21	0.07-0.62	0.005
Change in CXCR1	1.13	0.75-1.7	0.56	-	-	-

OR: Odds ratio, CI: Confidence interval, CXCR1: Chemokine receptor type 1.

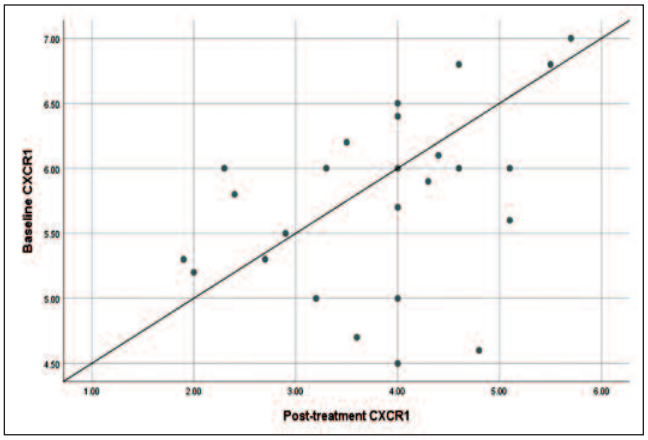

Figure: Oral manifestation in coronavirus disease-2019 (COVID-19) patients. A, B: Erosion in hard and soft palate. C, D, E: Ulcer in gingiva, labial mucosa and lip. F: Median rhomboid glossitis (Candida infection).

Discussion

The findings of the current study were in accordance with Xue et al.[6] and Chen et al.[9] which imply that the level of BC malignancy is correlated with CXCR1.

In the current study, patients with marked pathological response had significantly lower baseline and post-treatment CXCR1 expression, which is in agreement with Xue et al.[6]

On the basis of the findings, the therapeutic response may be enhanced if CXCR1 expression is suppressed using CXCR1 blockers.

Reparixin, a potent CXCR1 inhibitor, has been found to be helpful in lowering tumour development and recurrence[10].

The current study has limitations as the sample size was not calculated, which may have influenced the power of the study. Further studies are recommended to discover the effect of post-NAC CXCR1 expression.

Conclusions

There was an association between baseline and post-treatment CXCR1 expression in BC tissues and treatment response to NAC in BC patients.

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Conflict of Interest: None.

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