Correlation of Immunohistochemical Expression of CXCR4 With Clinicopathological Characteristics of Invasive Ductal Carcinoma of Breast

Anahita Nosrati[1], Zhila Torabizadeh[2], Mahboobeh Majlesi[3]

[1] *Department of Pathology, Gastrointestinal Cancer Research Center, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran*

[2] *Department of Pathology, Gut and Liver Research Center, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran*

[3] *Department of Pathology, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran*

Received: 02 Aug. 2017; Accepted: 18 Dec. 2017

Abstract- Breast cancer is the most frequent malignancy among women worldwide including Iranian women. The different markers have already been investigated for predicting invasion and metastases which one of the most tempting ones is Chemokines. In fact, one of the well-known mediators in the breast cancer metastases is stromal cell-derived factor-1 (SDF-1) CXCR4 chemokine. The CXCR4 chemokine receptor is a G-protein-coupled receptor that selectively binds to SFD-1 (also known as CXCL12). It is obvious that this chemokine receptor plays a critical role in some biologic processes. Nevertheless, there is not enough study to confirm the CXCR4 clinical importance and also its exact prognostic worth in the breast cancer. We carried out this diagnostic study. Immunohistochemically on 70 paraffin blocks of invasive ductal breast carcinoma and adjacent normal tissue simultaneously to assess the expression of CXCR4. The correlation between the presence and intensity of expression of this marker with various clinicopathological factors including age, tumor size, lymph node involvement, stage, and grade are evaluated in all patients. Among the 70 cases, 64 cancer specimens (91.4%) showed CXCR4 expression. It is found out that there is a significant difference between the expression of CXCR4 and the histological grade and lymph node metastasis (P<0.001), but no correlation with other clinicopathologic parameters, such as age, tumor size, and the stage is identified. By considering the CXCR4 intensity, we came across a significant difference between the high expression and the size, stage, histological grade and lymph node metastasis (P<0.001). Breast cancer is the most common invasive tumor to afflict women through the world. According to our study, there is a significant relationship between the expression of CXCR4 and grade, lymph node metastasis in the breast cancer. Furthermore, there is a direct significant correlation between the intensity of expression with grade, stage, tumor size, and lymph node metastasis. Thus, it can be used as a predictor factor for the breast cancer.

© 2018 Tehran University of Medical Sciences. All rights reserved.

Acta Med Iran 2018;56(6):366-371.

Keywords: Breast; Invasive ductal carcinoma; CXCR4; Clinicopathologic characteristics; Immunohistochemistry

Introduction

Cancer is on the rise in human population due to increase of elderly population, change of lifestyle and rise of bad habits such as consuming tobacco, unhealthy diet, limited physical activity, etc. (1). Breast cancer is the most common invasive tumor to affect women in the world, and the second cause of death in women with cancer etiology after lung malignancies (2). Breast cancer approximates 24.6% of total cancers in Iran of whom 97.1% are females. The average age of female patients is 49.6 years (3) while the average age of breast cancer diagnosis in western country is 56-year-old (4). A variety of risk factors affect breast cancer including endogenous and exogenous hormonal factors, lifestyle, familial history, and environmental contaminants (5).

Medically speaking, most important prognostic factors include involvement of lymph node, tumor size, histological grade, vessels and lymphatic invasion, ethnicity, and patient's age (6). Breast cancer is not fatal in initial stages but in the end progression and metastasis can cause 90% mortality. Overall survival for patient

Corresponding Author: Zh. Torabizadeh
Department of Pathology, Gut and Liver Research Center, Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran
Tel: +98 911 1513073, Fax: +98 11 33361706, E-mail address: zhtorabi@yahoo.com

without metastasis is 90% while metastasis will reduce it to 20% (7). Almost 10-15% of patients have aggressive disease and distant metastases within 3 to 10 years after initial diagnosis. However, this risk exists in lifetime. The risk of metastasis increases with lymph node involvement, increase in tumor size, and higher grade (8).

In most cases, patients visit physicians in advance stages due to lack of knowledge about symptoms of the disease, therefore although the prevalence of breast cancer is one-fifth of western countries, unfortunately, mortality rate is much higher (9).

The various markers are investigated for predicting of invasion and metastases of this tumor among them. Chemokines are one of the most tempting ones. Chemokines belong to a big family of small cytokine-like proteins, which do regulation of cytoskeletal and adhesion of endothelial cells and directional immigration (10). Signaling path of stromal cell-derived factor-1 (SDF-1) CXCR4 chemokine is a known mediator in some neoplasm and even in breast cancer which is shown by Muller *et al*., for the first time (11). CXCR4 chemokine receptor is a G-protein-coupled receptor which has selectively attachment to SDF-1 and is known as CXCL12. It is obvious that this chemokine receptor plays a critical role in variety of biological processes such as immunological cells hemostasis like T cell lymphocytes (12). CXCL12 has also been found to express in a variety of organs like the lungs, liver, bone marrow, kidneys, skeletal muscle, and brain. The organs which express high amount of CXCL12 are most common sites of secondary metastases (8). The potential mechanism of CXCR4 in progression and metastasis of tumor is induction of trans immigration of endothelial cells from initial site (13). Additional evidence confirms that not only CXCR4 is effective in breast cancer, but also it increases proliferation of tumor cells by increasing blood vessels (14). A group of studies showed a significant correlation between high expression of CXCR4 and invasive behavior of breast cancer (9,13) while other studies could not show this correlation (14-16).

Therefore, regarding considerable growth rate of breast cancer in different countries such as Iran and lack of proper medical response with the existence of controversial results, we aimed to study kind of provisional chemokine as CXCR4 immunohistochemically in patients with breast cancer. It stands to reason that there has not been enough study yet to confirm the CXCR4 clinical importance and also its exact prognostic worth in the breast cancer and so it seems that this work will be evaluable step to solve the existing controversies and to introduce a new prognostic marker for medical interventions.

Materials and Methods

This diagnostic study was conducted on 70 formalin-fixed paraffin-embedded blocks of breast infiltrative ductal carcinoma available at archives of Pathology department of Imam Khomeini Hospital, Sari, Iran during 2011-2016. Moreover, the adjacent normal-looking tissue is selected as control. All selected patients had not been subjected to chemotherapy and radiotherapy before surgery.

The clinicopathologic parameters, which were retrieved from the pathological database included age, tumor size, histological grade, lymph node metastasis, and stage are evaluated and compared with CXCR4 expression as well.

Immunohistochemical analysis

Initially, eligible paraffin blocks of breast infiltrative ductal carcinoma are removed from archives and 5 microns hematoxylin-eosin stained slides are prepared from the region of transition between tumoral-non neoplastic normal-looking tissues as target and internal control samples. Squamous cell carcinoma of cervical tissue and peripheral blood smear were used for positive and negative control of the marker respectively.

According to immunohistochemistry procedure for deparaffinizing and cleaning sections, prepared slides are incubated in 60º c for one hour at room temperature and then are put in xylene, absolute ethanol, and ethanol 96° respectively (2 times for 5 minutes, each time in one solution). Afterward, they should be washed by tap water and transferred to 1% hydrogen peroxide mixture (to eliminate internal peroxidase) and methanol, followed by the target solution after 10 minutes. For Antigen retrieval step, they are placed in a microwave oven for 13 min. up to boiling degree. After washing the slides with tap water and wash buffer, they are placed in a moist chamber and incubated by envisioning for 60 minutes using diagnostic kits CXCR4 antibody 1.200 dilution (Abcam). Then, they are washed two times with wash buffer. DAB solution is added, and after appearing brown color, they have placed again in wash buffer for 2 minutes. In the end, the washed slides are stained with Mayers Hematoxylin and mounted with Entellan glue.

Two expert and board-certified pathologists evaluated the prepared slides regarding expression and intensity of cytoplasmic and/or membranous CXCR4 expression, and results are reported semi-quantitatively.

Intensity of staining were reported as 4 scores: 0: lack

of staining, 1: weak, 2: moderate, 3: strong. Percentage of staining were reported as 4 scores: 1: 0-25%, 2: 26-50%, 3: 51-75%, 4: 76-100%. Staining index: (score of tumor cell staining)×(score of staining percentage) (Figure:1-4). Staining index≥6 was considered high and< 6 is considered low expression.

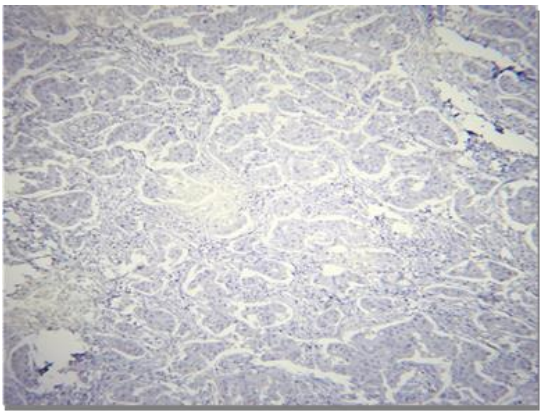

Figure 1. Negative IHC staining in tumoral cells (magnification ×100)

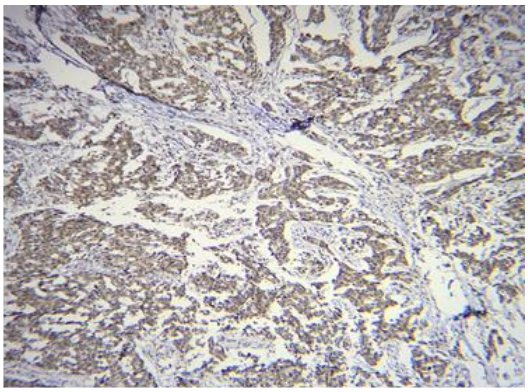

Figure 2. Mild IHC staining (1+) in the tumoral cells (magnification ×100)

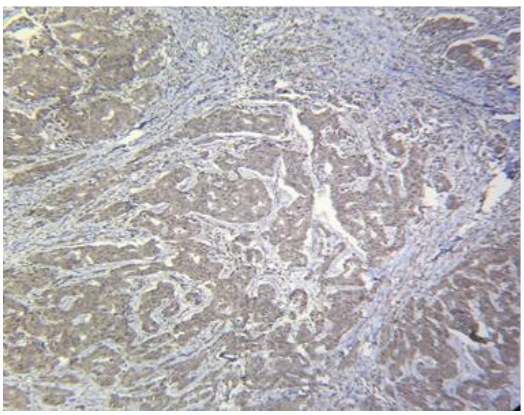

Figure 3. Moderate IHC staining (2+) in the tumoral cells (magnification ×100)

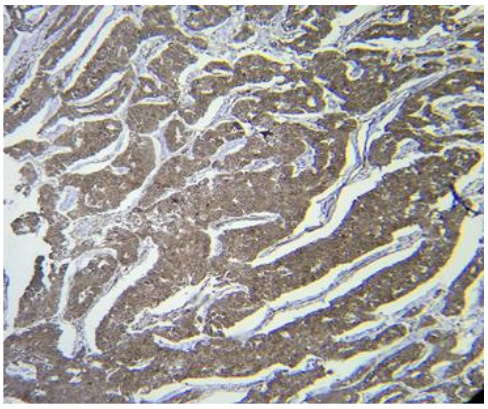

Figure 4. Severe IHC staining (3+) in the tumoral cells (magnification ×100)

Statistical analysis

Finally, the results were analyzed by statistical software SPSS (IBM SPSS Statistics 24). Chi-square, Fisher's exact tests, and MCnemar were used to analyze the correlation between the expression and intensity of expression of CXCR4 with clinicopathological parameters. A *P* less than 0.05 was considered as a statistically significant.

Results

We studied 70 cases with IDC of the breast in which most important clinicopathological characteristics are summarized in Table 1.

Table 1. Clinicopathologic findings in patients with breast cancer

		Percent	Number
Age	**≤50**	51.4%	36
	>50	48.6%	34
Tumor size	**<2 cm**	17.1%	12
	2-5 cm	61.4%	43
	5 cm<	21.5%	15
Histological grade	**1**	24.3%	17
	2	61.4%	43
	3	14.3%	10
Lymph node metastasis	**Positive**	58.6%	41
	Negative	41.4%	29
Stage	**1**	14.3%	10
	2	38.6%	27
	3	47.1%	33

In case of group, 65 (91.4%) cases showed positive staining, but in control group 43 (61.4%) displayed positive expression. Chi-square test was used in analyzing and comparing CXCR4 expression between two groups, and interestingly result showed a significant

difference between case and control groups (P<0.001).

By comparing CXCR4 marker expression with clinicopathological parameters, a significant correlation was statistically found between CXCR4 marker expression and histological grade and lymph node metastases, while there was no apparent correlation with age, tumor size, and stage. Table 2 shows correlation between clinicopathological parameters and CXCR4 expression.

By comparing the intensity of CXCR4 expression with clinicopathological parameters of patients with breast cancer and analyzing statistical data, a significant correlation was found between intensity of expression with the size of the tumor, histological grade, stage and lymph node metastases (Table 3).

Table 2. The correlation between biomarker CXCR4 expression and primary tumor characteristics

Clinicopathologic parameters		CXCR4 expression				*P*
		Positive		Negative		
		Number	Percent	Number	Percent	
Age	**≤50**	31	44.3%	5	7.2%	0.2
	50<	33	47.1%	1	1.4%	
Tumor size	**<2cm**	11	15.7%	1	1.4%	1
	2-5cm	39	55.8%	4	5.7%	
	5cm<	14	20%	1	1.4%	
Histological grade	**1**	12	17.2%	5	7.2%	0.007
	2	42	60%	1	1.4%	
	3	10	14.2%	0	0%	
Lymph node metastasis	**Positive**	41	58.6%	0	0%	0.004
	Negative	23	32.9%	6	8.5%	
Stage	**1**	8	11.4%	2	2.9%	0.14
	2	24	34.3%	3	4.3%	
	3	32	45.7%	1	1.4%	

Table 3. The correlation between the intensity of CXCR4expression and clinicopathologic parameters

Clinicopathologic parameters		CXCR4 intensity expression				*P*
		Low		High		
		Number	Percent	Number	Percent	
Age	**≤50**	11	17.2%	20	31.2%	0.94
	50<	12	18.8%	21	32.8%	
Tumor size	**<2cm**	9	14.1%	2	3.1%	0.001
	2-5cm	12	18.8%	27	42.1%	
	5cm<	2	3.1%	12	18.8%	
Histological grade	**1**	8	12.5%	4	6.2%	0.006
	2	14	21.9%	28	43.7%	
	3	1	1.6%	9	14.1%	
Lymph node metastasis	**Positive**	7	10.9%	34	53.2%	0.001
	Negative	16	25%	7	10.9%	
Stage	**1**	7	10.9%	1	1.6%	0.0001
	2	12	18.8%	12	18.8%	
	3	4	6.2%	28	43.7%	

Discussion

Chemokines have pivotal role in the development of immune system and may bind to different receptors, and basically, one receptor can bind to multiple chemokines. The CXCL12 chemokine binds to CXCR4 and CXCR7. CXCL12-CXCR4 pathway plays a critical role in homing of hematopoietic stem cells on bone marrow, lymphocytes trafficking, angiogenesis, and cell immigration (17).

CXCR4 is widely expressed in hematopoietic and other tissues, such as brain, lungs, colon, heart, kidney, liver and endothelial cells. The Expression of CXCR4 on malignant epithelial cells and on malignant hematopoietic cells implies that CXCL12-CXCR4 pathway can affect cancer's behavior and its metastases to organs which express high level of CXCL12 (18).

The intensity of expression of this marker can predict

invasions and metastases of breast cancer (19). High level of CXCR4 expression and its relation with tumor recurrence and metastasis in the large bowel (20), prostate (21), kidney (22), lungs (23), and pancreas (24) malignancies have also been reported.

Sun *et al*., and Liu *et al*., demonstrated CXCR4 expression rate on breast cancer 82% and 77% respectively (10,19). Furthermore, Holm and Kang *et al*., showed 100% positive staining (15,25). In our study, 91.4% of cases expressed CXCR4 marker. Actually, the difference in expression rates can be due to various staining methods or evaluating CXCR4 expression too.

A group of studies confirmed a significant correlation between high expression of CXCR4 and invasive behavior of breast cancer like poorly differentiation, lymph node metastasis and number of involved lymph nodes which was compatible with our study except we did not assess the number of involved lymph node (9,13) while other studies showed no significant correlation (14,15,16). In Sun *et al*., study a significant correlation was found between CXCR4 expression and stage of disease while no such correlation was found in our study (19).

Considering the tumor size, Sun *et al*., (19), Andre *et al*., (13), and Holm *et al*., (15) found no significant correlation between tumor size and CXCR4 expression. Similarly, in this study there was no significant statistical correlation between these two variables.

Sun *et al*., (19), Yasuoka *et al*., (14), and Holm *et al*., (15) did not find a significant correlation between CXCR4 expression and patient's age which was similar to our results.

Although it is not found statistically significant correlation between CXCR4 expression and tumor size and stage in our study, it is shown that high expression of this marker is significantly correlated with tumor size and most of the tumors with high expression are 2-5 centimeters in diameter. In addition, high expression of CXCR4 have direct relation with histological grade and lymph metastases, as it is displayed in other studies (10,13,25). In fact, we had some limitations due to short follow-up period, evaluating distant metastasis, recurrence and survival rate and so future studies are needed to work on recurrence tumors and survival are suggested as well.

To sum up, this study shows that there is a strong significant correlation between the presence and intensity of expression of CXCR4 with some clinicopathological factors of breast cancer including lymph node metastasis, histological grade, size, and stage of disease. Therefore, this biomarker can be used as a prognostic marker in predicting tumor behavior in clinical practice. To achieve more conclusive results, we recommend evaluating correlation between CXCR4 expression and survival rate, distant metastasis, and recurrence in further studies.

Acknowlegements

We thank Dr. Kamyar Khodadadi for discussion of ideas and Clinical research Development Unit Of Imam Khomeini Hospital, Mzandaran University of Medical Sciences, Sari, Iran for their support and helpful consultation.

References

1. Afsharfard A, Mozaffar M, Orang E, Tahmasbpour E. Trends in epidemiology, clinical and histopathological characteristics of breastcancer in Iran: results of a 17yearstudy. Asian Pac J Cancer Prev 2013;14:6905-11.
2. Ban KA, Godellas CV. Epidemiology of breast cancer. Surg Oncol Clin N Am 2014;23:409-22.
3. Jazayeri SB, Saadat S, Ramezani R, Kaviani A.Incidence of primary breast cancer in Iran: Ten-year national cancer registry data report. Cancer Epidemiol 2015;39:519-27.
4. Youlden DR, Cramb SM, Dunn NA, Muller JM, Pyke CM, Baade PD. The descriptive epidemiology of female breast cancer: an international comparison of screening, incidence, survival and mortality. Cancer Epidemiol 2012;36:237-48.
5. Arveux P, Bertaut A. Epidemiology of breast cancer.Rev Prat 2013;63:1362-6.
6. Cianfrocca M, Goldstein LJ. Prognostic and predictivefactors in early-stagebreast cancer. Oncologist 2004;9:606-16.
7. Mukherjee D, Zhao J. The Role of chemokine receptorCXCR4 in breast cancer metastasis. Am J Cancer Res 2013;3:46-57.
8. Weigelt B, Peterse JL, van't Veer LJ. Breast cancer metastasis: markers and models. Nat Rev Cancer 2005;5:591-602.
9. Enayatrad M, Amoori N, Salehiniya H. Epidemiology and trends in breast cancer mortality in Iran. Iran J Public Health 2015;44:430-1.
10. Liu Y, Ji R, Li J, Gu Q, Zhao X, Sun T, et al. Correlation effect of EGFR and CXCR4 and CCR7chemokinereceptors in predicting breast cancer metastasis and prognosis. J Exp Clin Cancer Res 2010;29:16.
11. Müller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. Nature 2001;410:50-6.

12. Hiller DJ, Meschonat C, Kim R, Li BD, Chu QD. Chemokine receptor CXCR4 level in primary tumors independently predicts outcome for patients with locally advanced breast cancer. Surgery 2011;150:459-65.
13. Andre F, Xia W, Conforti R, Wei Y, Boulet T, Tomasic G, et al. CXCR4 expression in early breast cancer and risk of distant recurrence. Oncologist 2009;14:1182-8.
14. Yasuoka H, Tsujimoto M, Yoshidome K, Nakahara M, Kodama R, Sanke T, et al. Cytoplasmic CXCR4 expression in breast cancer: induction by nitric oxide and correlation with lymph node metastasis and poor prognosis. BMC Cancer 2008;8:340.
15. Holm NT, Byrnes K, Li BD, Turnage RH, Abreo F, Mathis JM, et al. Elevated levels of chemokine receptor CXCR4 in HER-2 negative breast cancer specimens predict recurrence. J Surg Res 2007;141:53-9.
16. Andre F, Cabioglu N, Assi H, Sabourin JC, Delaloge S, SahinA, et al. Expression of chemokine receptors predicts the site of meta static relapse in patients with axillary node positive primary breast cancer. Ann Oncol 2006;17:945-51.
17. Xu TP, Shen H, Liu LX, Shu YQ. The impact of chemokine receptorCXCR4 on breast cancer prognosis: a meta-analysis. Cancer Epidemiol 2013;37:725-31.
18. Xu C, Zhao H, Chen H, Yao Q. CXCR4 in breast cancer: oncogenic role and therapeutic targeting. Drug Des Devel Ther 2015;9:4953-64.
19. Sun Y, Mao X, Fan C, Liu C, Guo A, Guan S, et al. CXCL12-CXCR4 axis promotes the natural selection of breast cancer cell metastasis. Tumour Biol 2014;35:7765-73.
20. Nosrati A, Torabizadeh ZH. Study of Chemokine Marker CXCR4 in Colorectal Cancer in Northern Iran. J Mazandaran Univ Sci 2016:25;60-68.
21. Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS, McCauley LK. Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. Cancer Res 2002;62:1832-7.
22. Pan J, Mestas J, Burdick MD, Phillips RJ, Thomas GV, Reckamp K, et al. Stromalderivedfactor-1 (SDF-1/CXCL12) and CXCR4 in renal cell carcinoma metastasis. Mol Cancer 2006;5:56.
23. Spano JP, Andre F, Morat L, Sabatier L, Besse B, Combadiere C, et al. Chemokine receptor CXCR4 and early-stage non-small cell lung cancer: pattern of expression and correlation with outcome. Ann Oncol 2004;15:613-7.
24. Maréchal R, Demetter P, Nagy N, Berton A, Decaestecker C, Polus M,et al. High expression of CXCR4 may predict poor survival in resected pancreatic adenocarcinoma. Br J Cancer 2009;100:1444-51.
25. Kang H, Watkins G, Douglas-Jones A, Mansel RE, Jiang WG. The elevated level of CXCR4 is correlated with nodal metastasis of human breast cancer. Breast 2005;14:360-7.