

Original Research Article

Expression of anaplastic lymphokine and HER2/neu immunostaining in patients with breast carcinoma

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ABSTRACT

Background: The diagnosis and prognosis of breast cancer is done with various immunomarkers including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2/neu), and Ki-67. The diagnostic utility of Anaplastic Lymphokine (ALK) and HER2/neu should be explored for better management of breast carcinoma patients. So, the present study was designed to determine the expression of immunostaining with ALK and HER2/neu in patients with breast cancer and to compare the association of ALK expression and HER2/neu with clinicopathological parameters.

Methods: This is a cross sectional multicenter study carried out from October 2016 to March 2017. A total of 140 subjects having breast carcinoma by using non-probability purposive sampling technique were selected. After taking informed consent, tissue samples were taken from received specimen of mastectomy for hematoxylin and eosin stain. The immunohistochemistry for ALK and HER2/neu were assessed on the paraffin blocks of the tumor.

Results: Out of total 140 cases invasive ductal carcinoma (89%), invasive lobular carcinoma (8%), invasive medullary carcinoma (2.1%) and papillary carcinoma (0.7%) were seen. ALK expression was positive in 81 patients (58%) while HER2/neu expression was positive in 53 cases (38%). Significant association was observed between ALK expression with histological grade, lymph node involvement, skin involvement and necrosis. There was also a significant association was seen between HER2/neu expression with lymph node metastasis and necrosis.

Conclusions: Present study shows higher expression of ALK when compared to HER2/neu expression in breast cancer patients.

Keywords: Anaplastic lymphokine, Breast cancer, Human epidermal growth factor receptor 2

INTRODUCTION

Breast carcinoma is second most common cause of women mortality. Almost 1.5 million newly diagnosed breast cancer females are reported around the globe every year.¹ A data base for the Indian and Pakistani female immigrants to United States for epidemiological incidences have reported younger less than 40 years females with increased risk of breast cancer. In a study conducted in Karachi revealed that about 33% of the total cancers in the females are breast cancer.² The age-related

incidence rate of breast cancer in Pakistani females is found to be around 50.1/100,000 population on yearly basis. The changes in reproductive and menstrual characteristics can influence the age-related breast cancer incidence as is found very high about 90 per 100,000 women in Western Europe where as comparatively very low 19.3 per 100,000 in Eastern African women.³

The most important risk factors of breast cancer are hormonal factors, lack of breast feeding, menarche at younger age, nulliparity, parity at older age have been

found to increase breast cancer risk.^{3,4} Breast cancer is caused by genetic and non-genetic factors usually involving the mutations in breast cancer susceptibility gene 1 (BRCA1) and breast cancer susceptibility gene 2 (BRCA2).^{5,6} For the purpose of breast cancer management and its prognosis, various markers including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (Her2/neu), and Ki-67 have been focus of researchers.⁷

HER2/neu, a growth factor receptor of tyrosine kinases (epidermal growth factor) family encodes HER2/neu, is placed on chromosome 17q11.2- q21. Her2/neu over expression exhibits to unregulated cell growth and may lead to oncogenic transformation. This molecular alteration is evident in various human cancers, like breast and ovarian cancer. Patients with HER2/neu over expression are associated with poor prognosis, shorter reoccurrence time, and decreased survival rate.^{8,9}

Anaplastic Lymphoma Kinase (ALK) a member of insulin receptor superfamily was reported in the year of 1994 for the first time as Nucleophosmin gene protein in patients with anaplastic large-cell lymphomas.¹⁰ This oncogenic variant of ALK is formed due to chromosomal translocation (2;5) (p23;q35) resulting in the abnormal release of NPM-ALK fusion protein responsible for ALK expression.¹⁰ During the intrauterine life brain and peripheral nervous system reveal the expression of ALK protein however its expression start diminishing after the birth among normal tissues.¹¹ Beside this ALK expression has been found in several malignancies including rhabdomyosarcoma, neuroblastoma, neuroectodermal tumor, glioblastoma and melanoma.¹² Thus the present study is designed to determine the expression of immunostaining with ALK and HER2/neu in patients with breast cancer and find out the association between expression of ALK and HER2/neu with clinicopathological parameters.

METHODS

The present cross-sectional study was conducted from October 2016 to March 2017. Sample size for the study was calculated at 23% probability and was calculated as 140 patients. The diagnosed female patients of all ages with breast carcinoma were included. The patients with metastatic breast cancer or who received chemo or radiotherapy were excluded. The ethical approval of the study was obtained from Institutional Research and Ethical committee. An informed consent was obtained either from the patient or her guardian. The biopsy samples were processed and stained with H&E. The ALK and HER2/neu immunostaining (IHC) was applied on all the biopsy samples which were confirmed as breast cancer on H&E stain.

The immunohistochemistry was carried out by using DAKO Monoclonal Mouse Anti-Human CD246 (ALK) Ab (DAKO, Denmark) according to manufacturer

protocol (13). Staining was graded semiquantitatively, as follows.

- 0 for absent or barely perceptible expression in rare cells.
- 1 for weak to moderate multifocal expression.
- 2 for strong staining in most cells.¹³

For HER-2/neu immunostaining; DAKO autoimmunostainer (DAKO, Carpinteria, CA) and its instruction were followed. Expression of Her2-neu status was evaluated using the Hercep Test™. The score was determined by immunohistochemical analyses (Hercep Test™). Score 0 means that no staining or membrane staining that is incomplete and faint in <10% of the tumor cells. Score 1+ is also negative and indicates a faint/barely perceptible membrane staining is detected in >10% of tumor cells. The cells exhibit incomplete membrane staining. A score 2+ is equivocal or weakly positive that indicates a weak-to-moderate complete membrane staining in >10% of the tumor cells. A score of 3+ by IHC was considered as positive and it shows circumferential homogenous dark staining in >10% of the tumor cells.¹⁴

RESULTS

A total of 140 female patients diagnosed with breast carcinoma were included in this study. Information regarding the cancer's characteristics as per Proforma-I was noted. The recorded data had information including age, size of tumor, laterality of tumor, histological type, histological grade, involvement of skin, vascular invasion, presence or absence of necrosis, lymph node involvement and lastly menopausal history. Immunohistochemistry for the assessment of ALK and Her2/Neu was done with microscopic examination. The recorded results were then tested for association with different parameters of breast cancers. An association with a p-value of ≤ 0.05 was taken to be of significance. Majority of patients, 81 (57.9%) were found to be positive for ALK. Negative ALK was reported in 59 (42.1%) of patients (Table 1, Figure 1 and 2). Positive HER2/neu was recorded in 53 (37.9%) of patients. Higher number of patients, 87 (62.1%) were reported negative for HER2/neu (Table 1, Figure 3).

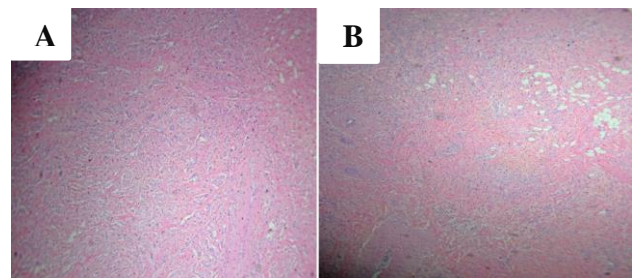


Figure 1: H & E (100x) staining in invasive ductal carcinoma, (A): and invasive lobular carcinoma, (B): of breast cancer.

Table 1: Expression of ALK and HER2/neu immunostaining in patients with breast cancer.

Expression	Frequency of ALK	Frequency of HER2/neu
Negative	59 (42.1%)	87 (62.1%)
Positive	81 (57.9%)	53 (37.9%)
Total	140 (100%)	140 (100%)

ALK association with various parameters

Among all patients, highest number 73 (58.4%) had invasive ductal carcinoma positive for ALK, while 52 (41.6%) patients were negative for ALK. Out of 89

patients, 58 (65.2%) patients were positive while 31 (34.8%) patients were negative for ALK for lymph node involvement. A significant p-value of 0.021 was recorded. Skin involvement and necrosis with positive expression of ALK was found in 40 (69%) and 43 (67.2%) patients respectively with significant p-values. However vascular invasion showed positive expression in 29 (61.7%) patients with non-significant p-value of 0.512. Regarding histological grades, 45 (76.3%) had grade-III, 35 (48.6%) in grade-II and only 1 (11.1%) patient in grade-I were positive for ALK with highly significant p-value of 0.001 (Table 2).

Table 2: Association of ALK with different clinicopathological parameters.

Clinicopathological parameters		Alk (n=140)		Total (n=140)	P-value
		Negative (59 cases)	Positive (81 cases)		
Histological type	Invasive papillary Ca	0 (0.0%)	1 (100.0%)	1 (100.0%)	0.675
	Invasive ductal Ca	52 (41.6%)	73 (58.4%)	125 (100.0%)	
	Invasive lobular Ca	6 (54.5%)	5 (45.5%)	11 (100.0%)	
	Invasive medullary Ca	1 (33.3%)	2 (66.7%)	3 (100.0%)	
Lymph node	Negative	28 (54.9%)	23 (45.1%)	51 (100.0%)	0.021
	Positive	31 (34.8%)	58 (62.5%)	89 (100.0%)	
Skin involvement	Negative	41 (50.0%)	41 (50.0%)	82 (100.0%)	0.025
	Positive	18 (31.0%)	40 (69.0%)	58 (100.0%)	
Vascular invasion	Negative	41 (44.1%)	52 (55.9%)	93 (100.0%)	0.512
	Positive	18 (38.3%)	29 (61.7%)	47 (100.0%)	
Necrosis	Negative	38 (50.0%)	38 (50.0%)	76 (100.0%)	0.04
	Positive	21 (32.8%)	43 (67.2%)	64 (100.0%)	
Histological grade	I	8 (88.9%)	1 (11.1%)	9 (100.0%)	0.001
	II	37 (51.4%)	35 (48.6%)	72 (100.0%)	
	III	14 (23.7%)	45 (76.3%)	59 (100.0%)	

Table 3: Association of HER2/Neu with different clinicopathological variables.

Clinicopathological parameters		Her2/neu (n=140)		Total (n=140)	P-value
		Negative (59 cases)	Positive (81 cases)		
Histological type	Invasive papillary Ca	0 (0.0%)	1 (100.0%)	1 (100.0%)	0.369
	Invasive ductal Ca	80 (64.0%)	45 (36.0%)	125 (100.0%)	
	Invasive lobular Ca	6 (54.5%)	5 (45.5%)	11 (100.0%)	
	Invasive medullary Ca	1 (33.3%)	2 (66.7%)	3 (100.0%)	
Lymph node	Negative	26 (51.0%)	25 (49.0%)	51 (100.0%)	0.039
	Positive	61 (68.5%)	28 (31.5%)	89 (100.0%)	
Skin involvement	Negative	48 (58.5%)	34 (41.5%)	82 (100.0%)	0.269
	Positive	39 (67.2%)	19 (32.8%)	58 (100.0%)	
Vascular invasion	Absent	53 (57.0%)	40 (43.0%)	93 (100.0%)	0.047
	Present	34 (72.3%)	13 (27.7%)	47 (100.0%)	
Necrosis	Negative	43 (56.6%)	33 (43.4%)	76 (100.0%)	0.139
	Positive	44 (68.8%)	20 (31.3%)	64 (100.0%)	
Histological grade	I	6 (66.7%)	3 (33.3%)	9 (100.0%)	0.426
	II	41 (56.9%)	31 (43.1%)	72 (100.0%)	
	III	40 (67.8%)	19 (32.2%)	59 (100.0%)	

HER2/Neu association with different variables

Out of 53 positive HER2/neu cases, maximum 45 (36%) patients had invasive ductal carcinoma and 5 (45.5%) patients had invasive lobular carcinoma. Lymph node involvement and vascular invasion with positive expression of HER2/neu was found in 28 (31.5%) and 13 (27.7%) respectively with significant p-values. However, skin involvement and necrosis showed positive expression in 19 (32.8%) and 20 (31.3%) respectively with non-significant p-values.

Regarding histological grades, 31 (43.1%) had grade-II, 19 (32.3%) in grade-III and only 3 (33.3%) patient in grade-I were positive for HER2/neu with non-significant p-value of 0.426 (Table 3).

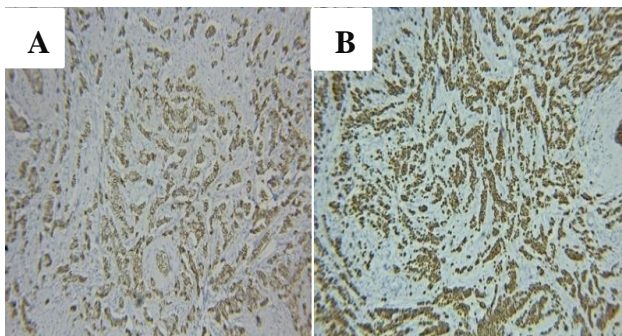


Figure 2: Immunohistochemistry of ductal carcinoma. (A): and lobular carcinoma, (B): with ALK positive (400x).

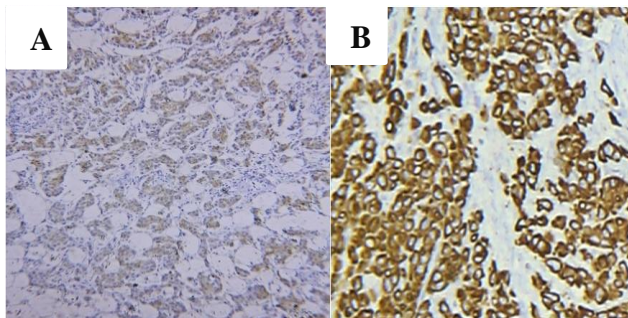


Figure 3: Immunohistochemistry of ductal carcinoma. (A): and lobular carcinoma, (B): with Her2/neu positive (400x).

DISCUSSION

Breast carcinoma is a disease with a tremendous heterogeneity in its clinical behavior and pathological variables such as tumor size, histologic grade, histological type, lymph node metastases, vascular space invasion, tumor cell proliferation and tumor necrosis.¹⁵ The present study was conducted to determine the expression of immunostaining with ALK and HER2/neu in patients with breast cancer and to know the association between

expression of ALK and HER2/neu with various clinicopathological parameters.

In present study invasive ductal carcinoma was observed in 125 (89.3%) patients, invasive lobular carcinoma and invasive medullary carcinoma was seen in 11 (7.9%) and 3 (2.1%) patients, whereas invasive papillary carcinoma was found in only 1 (0.7%) case. However Shankar et al, showed a similar figure in different histological types invasive ductal carcinoma was observed in 90.9%, invasive lobular carcinoma 6.76% and invasive medullary carcinoma in 1.87% and invasive papillary carcinoma was seen in 0.18% patients.¹⁶ Similar results were also reported by Mehrjardi et al.¹² Invasive ductal carcinoma was observed in 88%, invasive lobular carcinoma found in 8% and invasive medullary carcinoma in 4% of the breast cancer patients in his study.

In this study, positive cases of ALK was observed in 81 (57.9%) patients and negative in 59 (42.1%) patients whereas, Mehrjardi et al, reported 47% positive cases and 53% negative cases.¹² Positive expression of HER-2/neu was seen in 53 (37.9%) patients and negative in 87 (62.1%) patients. In comparison with Mahmoud reported 57.9% positive cases and 41.3% negative cases.¹⁷ Dayal et al, showed positive HER2/neu in 21.3% patients and 78.7% negative patients.¹⁸ Devi et al, presented 12.1% positive cases and 63.4% negative HER2/neu patients.¹⁹ Similar data were observed in Ambroise et al, research that 27.1% with positive expression of HER2/neu and negative in 87% subjects.²⁰ Contrary findings were documented in Dutta et al, study in which 57.3% HER2/neu positive cases were detected in breast cancer patients and 42% in negative cases were perceived.²¹ Munjal et al, reported consistent results with 40.2% HER2/neu positive cases and 59.8% negative patients.²²

In this study, significant association ($p < 0.05$) was found between skin involvement, lymph node involvement, necrosis and histological grade when compared with ALK, whereas histological type and vascular invasion were found to be insignificant when compared with ALK positivity. In the present study, invasive ductal carcinoma was found in 73 (58.4%), invasive medullary carcinoma in 5 (45.5%) cases and 2 (66.7%) case of invasive lobular carcinoma were present showing positive ALK expression with insignificant p-value of 0.675. Similar results in relation to ALK are reported in Mehrjardi et al, study.¹² Patient reported histological type, invasive ductal carcinoma in 43 (48.9%), invasive medullary carcinoma in 4 (50%) cases and no case of invasive lobular carcinoma was presented with insignificant p-value of 0.116. In the current study, positive cases of necrosis were noticed in 43 (67.2%) patients with ALK (significant p-value 0.04). In contrast to the present study findings Mehrjardi et al, reported positive cases of necrosis were seen in 9 (37.4%) patients when compared with ALK (insignificant p-value 0.244).¹² In this study,

vascular invasion was insignificant (p-value 0.512) observed with ALK 29 (61.7%) whereas similar results were reported by Mehrjardi et al, with insignificant P-value of 0.728 and the positive cases were 22 (50%).¹²

A significant association (p-value <0.05) was detected between lymph node and vascular invasion with HER2/neu patients. Whereas histological type, skin involvement, necrosis and histological grade in relation with HER2/neu were found to be insignificant. In this current study lymph node was found to be positive in 28 (31.5%) HER2/neu positive patients with significant p-value of 0.039. In comparison to present study, a study by Hashim et al, reported insignificant association (p-value 0.056) between lymph node and positive cases of HER2/neu.²³ Another study by Ali et al, showed similar results with significant association (p-value 0.001) found in lymph node positive 34 (13.2%) cases.²⁴ In the present study, regarding histological grade-I in relation to HER2/neu was found in 3 (33.3%), grade-II in 31 (43.1%) and grade-III in 19 (32.2%) with insignificant p-value of 0.426. In contrast to the findings of present results, study by Hashim et al, reported significant association (p-value 0.000) was seen with histological grade-I 5 (2.6%), grade-II 114 (58.2%) and in grade-III 77(39.3%) patients.²³ Another study by Sheikh et al, showed similar results with present study that no significant association (p-value 0.465) was found in different histological grades with HER2/neu in which grade-I was found in 5 (71.4%), grade-II in 47 (68.1%) and grade-III in 24 (57.1%) patients.²⁵ In this study, skin involvement in relation with HER-2/neu was present in 19 (32.8%) patients with insignificant p-value of 0.269. Similar results were reported in Sheikh et al, study reporting positive cases with skin involvement in 34 (66.7%) cases with insignificant P-value 0.635.²⁵ However in contrast results are reported in a study by Faheem et al, that 246 (51.46%) positive cases of skin involvement were seen with significant p-value of 0.001.⁶ In the view of present study findings recommended that breast malignancy in Pakistani females may be of aggressive tumor parameter, so ALK immunostaining may be used along with commonly used HER2/neu immunostaining for better therapeutic and prognostic approach.

CONCLUSION

Present study shows higher expression of ALK when compared to HER2/neu expression in breast cancer patients. Present study reveals significant association of ALK with histological grade, lymph node involvement, skin involvement and necrosis. Present study also found significant association between HER2/neu expression with lymph node involvement and vascular invasion.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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