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IMMUNOSTAINING STATUS OF ANAPLASTIC LYMPHOKINASE IN FEMALE BREAST CANCER.

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ABSTRACT… Objectives: To determine the status of ALK immunostaining in female breast cancer and to find out the relationship of ALK expression with clinicopathological variables. **Study Design:** Cross Sectional study. **Setting:** Department of Pathology Al Tibri Medical College and Hospital Karachi and BMSI Jinnah Post Graduate Medical Centre, Karachi. **Period:** October 2016 to March 2017. **Material & Methods:** Total of 110 female cases with breast carcinoma by using non-probability purposive sampling technique. After taking informed consent from the patients, tissue samples were taken from received specimen of mastectomy for hematoxylin and eosin stain. The immunohistochemistry for ALK was assessed by using a DAKO monoclonal antibody using the paraffin embedded blocks of the diagnosed cases of breast carcinoma. Chi square test was used to find out the significance of differences among the variables. **Results:** Total of 110 cases with different histological classifications of breast cancer were observed i-e invasive ductal carcinoma 95(86%), invasive lobular carcinoma 9(8%), invasive medullary carcinoma 4(3.6%) and papillary carcinoma 2(1.8%). ALK expression was positive in 71(64%) and negative in 39 (35%) patients. Significant association was observed between ALK expression with histological grade (p=0.003), lymph node involvement (p=0.042) and skin involvement (p=0.025). **Conclusion:** Present study shows higher positive ALK expressions. Present study also indicates significant relationship of ALK with histological grade, lymph node involvement and skin involvement.

Key words: Anaplastic Lymphokinase, Breast Carcinoma, Immunohistochemical Expression.

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INTRODUCTION

Tumors of breast are most widely recognized malignancy among females globally there after skin disease. Breast tumor counts about 16% out of complete diseases among females. The frequency is double to colorectal growth and cervical tumors. Throughout globe, the rate of breast tumor varies. Frequencies are lower among low-economic nations but higher among high-economic nations. Breast tumors are identified in just 5% of total malignancies of breast in women under age 40.[1] Females in all ages can be affected by cancer, in elderly, youth population, poor and rich women. It is causing budget related and social issues among Pakistani families. Pakistan is low economic nation and majority populations are residing in rustic territories. In Asia, Pakistan has one of the higher rate with breast cancer. High grade breast cancers with bad prognosis are reported in young females.[2] Financial issues cause high morbidity rate in Pakistani women. At some phase of life, 1 in 9 Pakistani women is found with breast carcinoma.[3] The frequency of breast growth was 30-40 in percentage among women in 40-year age range in Pakistan as associated with 10 for each in similar age groups in the West.[4] One of the most astounding frequency rates in Asia. Pakistani women demonstrate a rate of 50/100,000 and in the neighboring nation India; the frequency rate is 19/100,000.[5] The breast malignancy etiology aren't fully understood, leading to difficulty in suggesting the reason as to why a lady might produce breast tumor while some other might not. Some specific risk factors of breast cancer are ageing, family history, previous breast nodules,

hormonal factors, uses of birth control pills, alcoholism, obesity and radiations exposure.[6] Breast tumors are partitioned in subclasses having vital ramifications of prognosis and cure. Pathogenesis represents to chromosomal modifications found within various breast tumor variants as well as to detect particular gene that promote morbidity. mRNA expression levels in estrogen receptors, progesterone receptors as well as HER2 have appeared into future indicator through immunohistochemistry.[7]

A tyrosine kinase receptor known as Anaplastic lymphoma kinase (ALK), dwelling on chromosome 2p23 was first depicted in a subgroup of anaplastic large cell lymphoma (ALCL) subjects as a major aspect of chromosomal modification with nucleophosmin. ALK has been accounted to be translocated with other combination, for example KIF5B, NPM1, RET, ROS, VCL, TFG, EML4 and MYH9, showing its part in the carcinogenesis of different tumors. Moreover, different results show extra methods of constitutively initiated ALK kinase by mutations and ALK gene enhancement. It has been recommended that the tumor producing part of ALK is most presumably intervened by means of stimulation of tyrosine kinases that advance survival by means of activation through its route for example PI3-kinase/AKT or by hindrance of apoptosis, in this manner prompting multiplication of cells. It has been exhibited that ALK suppression represses development of breast cancer cell lines and furthermore tumor xenografts in models of mouse.[8]

MATERIAL & METHODS

The current study was cross-sectional and the duration was from October 2016 to March 2017. All the female patients diagnosed with breast cancer of all ages were included in the study. Metastatic breast carcinoma patients or those were on chemical and radiation treatment was excluded from the study. The ethical approval for the study was taken from Institutional Research and Ethical committee. An informed consent was taken from the patient or her guardian. The biopsy samples were prepared and stained with H&E. The ALK immunostaining (IHC) was processed on all the biopsy samples which were diagnosed breast cancer on H&E stain.The immunohistochemistry analysis was carried out by using DAKO Monoclonal Mouse Anti-Human CD246 (ALK) Ab (DAKO, Denmark) with the following instructions;

Tissue sections from paraffin-embedded blocks were collected on clean poly L-lysine coated glass slides, deparaffinized via oven for 1-2 hours at 60 °C and then immersed by three washing steps of xylene till 10 minutes separately, in two washes of 100% ethanol for 5 minutes respectively and in two washes of 95% ethanol till 5 minutes each. Then they was immersed twice in distilled water (dH2O) for 5 minutes individually, and was immersed in hydrogen peroxide 1/10 with methanol for 15 minutes. The slides were rinsed two times for 5 minutes as well as washed in Tris buffered saline (TBS) solution, containing 160gm NaCl, 12gm Tris (hydroxymethyl) and 100cc distilled water with pH adjusted to 7.6 in concentrated HCl for 5 minutes. Slides were sited within EDTA solution (0.372 gm EDTA ($C_{10}H_{14}N_2O_8Na_2 \cdot 2H_2O$) to 1 L dH_2O, pH adjusted to 8.0) into the pressure cooker, set for the Biocare Medical Decloaking Chamber follow (SP1 125°C 30 seconds and SP2 90°C 10 seconds). The slides were then let to cool on the bench for 10 minutes. Slides were rinsed with dH_2O. 100–300 µl primary antibody diluted according to the product quide (dilution ratio: 2/100, pH:6) and added to each slides, immersed for 40 minutes. Antibody solution removed by placing sections in wash buffer thrice till 5 minutes Upon process development, slides was immersed in dH_2O, counterstained in hematoxylin for 15 seconds and washed in dH_2O twice for 5 minutes each. In the Last complete dehydration was done with alcohol, cleared in xylene and mounted.[9]

Staining evaluation was as follows

1. for absent or barely perceptible expression in rare cells
2. for weak to moderate multifocal expression
3. for strong staining in most cells.[10]

RESULTS

Total 110 female diagnosed patients having cancer of breast were selected in this study. The

data recorded as laterality of tumor, histological type, tumor grade, involvement of skin, lymph node involvement and lastly menopausal history. Immunohistochemistry for the assessment of ALK was done with microscopic examination.

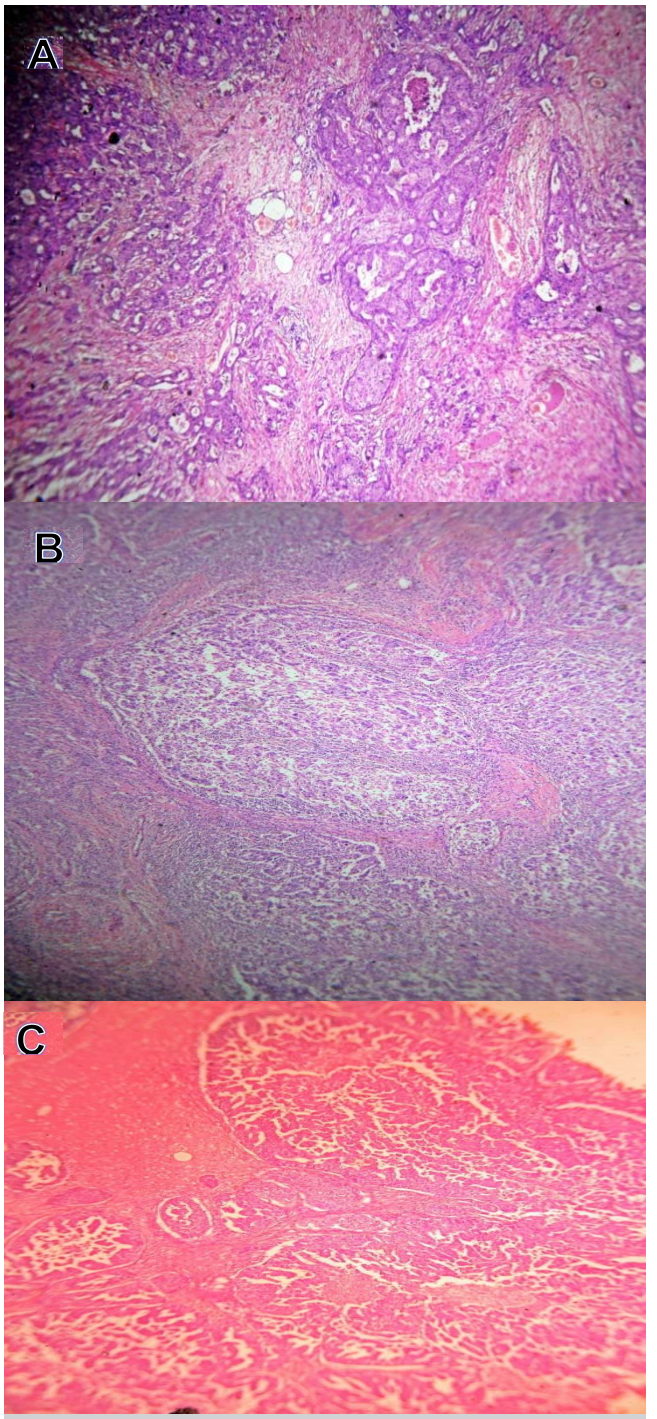

Figure-1. (A) H & E Staining of invasive ductal carcinoma (B) H & E Staining of Medullary carcinoma (C) H & E Staining of Papillary Carcinoma. (100x)

The documented results were then evaluated for association with different variables of breast cancers. An association with a P-value of≤ 0.05 was taken to be of significance. Most common positive expression of ALK was observed in 71 (64.5%) patients and negative in 39 (35.4%) patients (Table-I). 2(1.8%) patient had invasive papillary carcinoma, 95(86%) had invasive ductal carcinoma, 9(8%) patients had invasive lobular carcinoma and 4(3.6%) had invasive medullary carcinoma with non-significant P-value of 0.565 was recorded (Table-II).

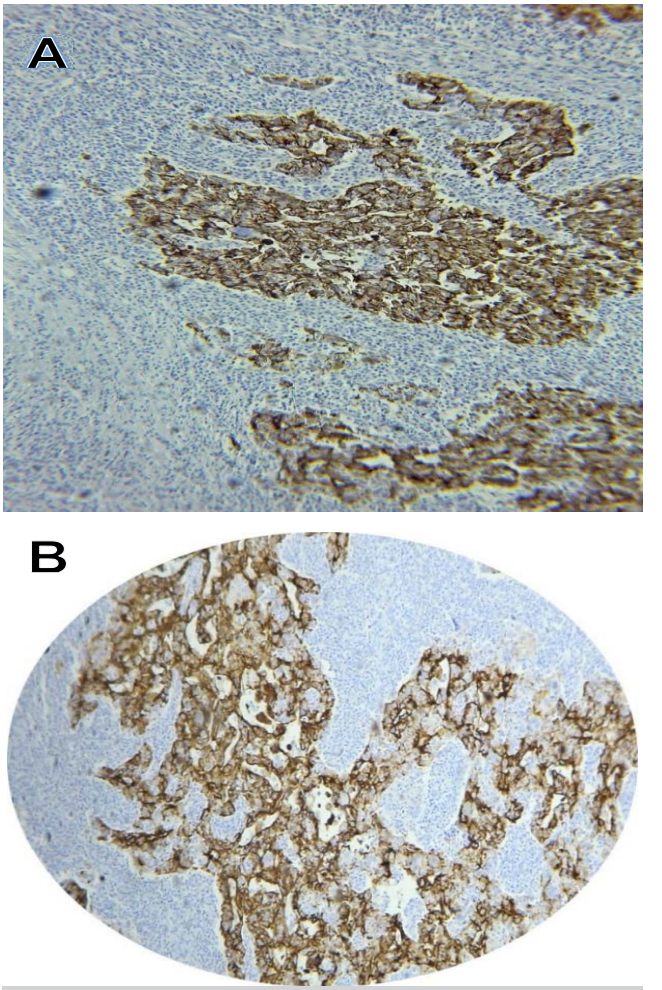

Figure-2. (A) Positive expression of ALK in Ductal Carcinoma (B) Positive expression of ALK in Medullary Carcinoma (100x)

Among 39 ALK negative patients, 23 (56.1%) had no lymph nodes involvement while 21 (30.4%) patients were positive lymph nodes. From the 71 ALK positive patients, 18 (43.9%) were negative for lymph nodes while 48 (69.5%) patients

had lymph nodes involvement with significant P-value of 0.042. Out of 39 patients negative for ALK, 7 (77.7%) patients had histological grade I, 26 (51.4%) patients had histological grade II, 10 (19.6%) patients had histological grade III. Amongst the 71 patients positive for ALK, only 2 (22.2%) patient had grade I histology while 24 (48.6%) had grade II histology and 41 (80.3%) had grade III histology with significant P-value of 0.003. A total of 39 ALK negative patients, 33 (77.1%) patients were negative skin involvement while 8 (20.0%) patients were positive for involvement of skin. Out of the 71 ALK positive patients, 37 (52.8%) patients were negative for skin involvement while 32 (80.0%) patients were positive for skin involvement with significant P-value of 0.025. From the 39 ALK negative patients, 29(46.7%) patients had tumor in left breast, 17 (36.9.7%) on the right side while none (0%) of the patient had bilateral breast tumor. Among the 71 ALK positive patients, 33(53.2%) patients had tumor on their left side, 29(63%) on the right side and 2 (100%) patients had bilateral breast tumor. Non-significant P-value of 0.107 was reported.

ALK	Frequency	Percent
Negative	39	35.4
Positive	71	64.5
Total	110	100.0

Table-I. Status of ALK in breast cancer

Variables		Alk (n=110)		Total	P-Value
		Negative (39 cases)	Positive (71 cases)		
Menopause	Pre menopausal	33	36	69	0.092
		47.8%	52.1%	100.0%	
	post menopausal	12	29	41	
		29.2%	70.7%	100.0%	
histological Type	invasive papillary Ca	0	2	2	0.565
		0.0%	100.0%	100.0%	
	Invasive ductal Ca	31	64	95	
		32.6%	67.3%	100.0%	
	invasive lobular Ca	5	4	9	
		55.5%	44.4%	100.0%	
	invasive medullary Ca	1	3	4	
		25.0%	75.0%	100.0%	
Tumor laterality	Left	29	33	62	0.107
		46.7	53.2%	100.0%	
	Right	17	29	46	
		36.9%	63.0%	100.0%	
	Bilateral	0	2	2	
		0.0%	100.0%	100.0%	
		36.0%	64.0%	100.0%	
lymph Node	Negative	23	18	41	0.042
		56.1%	43.9%	100.0%	
	Positive	21	48	69	
		30.4%	69.5%	100.0%	
skin Involvement	Negative	33	37	70	0.025
		47.1%	52.8%	100.0%	
	Positive	8	32	40	
		20.0%	80.0%	100.0%	
Histological grade	I	7	2	9	0.003
		77.7%	22.2%	100.0%	
	II	26	24	50	
		51.4%	48.6%	100.0%	
	III	10	41	51	
		19.6%	80.3%	100.0%	

Table-II. Relationship of ALK with menopausal status, histological type, tumor laterality, lymph node, skin involvement & histological grade

DISCUSSION

Breast malignancy is one of the most commonest carcinoma around the globe instead of progress in cancer management, breast cancer enhances a main causative factor in spread of different breast lesions and death in patients having breast malignancies.[11] Different studies reveal that ALK tyrosine kinase receptor is a reliable immunomarker and a good therapeutic predictor in managing a large number of breast malignancies.[12,13] Hence, in order to learn more on the prevalence and clinical significance of ALK overexpression and its association with clinical parameters in Middle Eastern breast cancer, we comprehensively investigated protein expression of ALK In the current study, pre-menopausal status was seen in 69 (62.7%) whereas post-menopausal cases were 41 (37.2%). Shankar A et al[14] reported 25.7% were pre-menopausal and 74.3% post-menopausal females in his study. Whereas Verbeek FPR et al[15] reported pre-menopause status in 26% patients and post-menopause status in 74% of the patients.

Current research reveals the histological type as invasive ductal carcinoma was observed in 95(86.3%) patients, invasive lobular carcinoma and invasive medullary carcinoma was seen in 9(8.1%) and 4(3.6%) patients, whereas invasive papillary carcinoma were found in only 2(1.8%) cases. However Shankar A et al[14] 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.

Similar results were also reported by Mehrjardi AZ et al.[9] 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. Regarding tumor laterality, in most cases of breast cancer 62 (56.3%) patients left breast involvement was seen, however 46 (41.8%) had right breast involvement while only 2(1.8%) were found with bilateral breast involvement. Similar results are showed by Mahmoud MM et al[16] that left breast was found involved in 75 (54.3%) patients, right breast was involved in 58 (42%) and bilateral tumor was observed in 5 (3.7%) patients. In this study positive lymph node was ssen in 69 (62.7%) cases and 41 (37.7%) were seen negative. Similar results are reported by Devi PU et al[17] that positive lymph node was found in 53.6% patients and negative lymph node was observed in 46.3%.

In current study, skin involvement with tumor tissue was noticed in 40 (36.3.%) and skin free of tumor tissue was seen in 70 (63..6%) patients. In terms of skin involvement Mahmood H et al[18] reported similar results 46.4% positive skin involvement whereas no skin involvement was seen in 53.6% of the cases. In the present study histological grade-I was found in 9 (8.1%) patients, grade-II in 50 (45..4%) and grade-III was seen in 51 (46.3%) cases. Mehrjardi AZ et al[9] also showed same results with the recent study that grade-I was observed in 7.6% patients, grade-II in 31.5% and grade-III was noticed in 60.9% of the patients. In Li Ding[19] study similar observations were noticed with histological grade-I in 7.6% cases, 53% in grade-II and 38% in grade-III.

In this study, positive cases of ALK was observed in 71 (64.5%) patients and negative in 39 (35.4.1%) patients whereas, Mehrjardi AZ et al[9] reported 47% positive cases and 53% negative cases. In this study, significant association ($P < 0.05$) was found between histological grade, Lymph node involvement and skin involvement when compared with ALK., whereas menopausal status, histological type, tumor laterality were found to be insignificant when compared with ALK positivity. Current study reveals significant association of histological grade with ALK (P-value 0.003). Similar result was found in Siraj AK et al[20] study with significance (P-value 0003).

CONCLUSION

In conclusion, immunostaining expression of ALK was investigated and the relationship of immunomarker ALK with different clinicopathological variables was studied. Present study indicated significant ALK relationship with histological grade, lymph node involvement and skin involvement.

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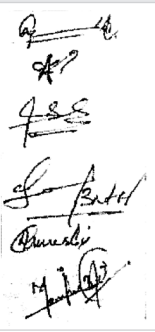
1	Syed Naqeeb Ali	Writing of the article and Final approval of article.	
2	Syed Liaquat Ali	Analysis and interpretation of data.	
3	Ghazala Panhwar	Drafting of the article.	
4	Sarwat Batool	Statistical expertise.	
5	Anila Qureshi	Critical revision of the article for major corrections.	
6	Momina Naseer	Data collection.	