Research Article

Correlation between Ki-67 index and Her-2 Expression Status in Invasive Ductal Carcinoma of Breast

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Abstract:

Background: Ki-67 index has been used to categorize primary infiltrating breast carcinoma into their surrogate molecular subtypes for selection of treatment strategies. HER-2 also serves as basis for molecular classification of breast cancer and patients having positive expression of HER-2/neu have been selected for anti-Her-2 therapy and have a better prognosis. In this article, we learn about 10 pictures to find the association of Ki-67 index and expression status of HER-2 in primary breast ductal carcinoma of infiltrating type. This correlation may improve insight into the prognosis of invasive ductal breast cancer and also play a vital role in therapeutic management of patients.

Methods: 143 primary ductal breast carcinoma cases having infiltrating type were analyzed at Pathology department in Fatimah Jinnah Medical University, Lahore between April 2019 and January 2020. All patients diagnosed on Tru-Cut/core, wedge biopsies and mastectomy specimens were included. Evaluation of Her-2 status and Ki-67 index was carried out by immunohistochemistry after routine formalin fixation and paraffin embedding of sections from tumor area.

Results: High index of Ki-67 was observed (> 14 %) in 74 cases (51.4%) and low (≤ 14%) in the remaining 69 (49.6%) cases. 79 cases (55.2%) were Her-2 positive and 64 cases (44.8%) were Her-2 negative. A significant (p value < 0.05) relationship was demonstrated between higher Ki-67 index and positive Her-2 expression.

Conclusion: This study concluded that strongly significant association is exists among high index of Ki-67 and positive Her-2 expression status.

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Introduction:

Breast Cancer has been regarded as one of top-ranked malignancies and causes of cancer-related deaths in females worldwide.[1] The age-standardized incidence rates of breast cancer in females are high in Pakistan.[2] The high mortality rate has led to ponder in the different aspects of this disease, including risk, etiology, predictive factors, prognosis, treatment modalities and management outcome.[3]

Following prognostic factors for breast cancer patients are used including grade of tumor, size of tumor, estrogen receptor (ER) expression, progesterone receptor (PR) status, involvement of axillary lymph nodes, status of Ki-67 proliferation rate and human epidermal growth factor receptor-2 (HER-2) expression.[4] The molecular classification of breast cancers has further elaborated the multi-factorial approaches towards breast cancer treatment. Ki-67 and Her-2 receptor expression status has independent importance for the prognosis of breast cancer.[5]

In early 1980's, Ki-67 antigen was identified as non-histone and labile nuclear protein. At long arm of chromosome 10, at location of 25-ter harbors gene of Ki-67. Ki-67 is associated with cell proliferation and its expression is variable during entire cell cycle which is at peak in mitosis. Ki-67 is the most widely used proliferative marker because cell cycle is regulated by it

and rate of cell proliferation is depicted by it. Data shows expression of Ki-67 in all active phases of cell cycle. Normal breast tissues have expressed a low level of Ki-67 i.e. less than 3% ductal epithelial cells show ER nuclear staining in cases having negative estrogen receptor (ER) status. Moreover, use of Ki-67 as biomarker for assessing as a growth fraction in a given population of cells, as well as, its role to estimate growth fraction of cell population undergoing neoplastic alterations is well established, especially when evaluated by using monoclonal Ki-67 antibodies. Ki-67 is observed as a vital factor for planning strategies for adjuvant treatment of breast ductal carcinoma. Ki-67 being an acknowledged proliferative marker has been an important and independent predictive and prognostic factor in breast carcinoma.[4] The rate of proliferation of tumor cells in invasive ductal breast cancer is directly related to percentage of cells showing positivity with Ki-67 immunohistochemistry in nuclei of dividing cells.[5,6] Invasive ductal breast carcinomas with higher Ki-67 proliferative index are directly linked to a higher grade and worse prognosis.[7]

As Ki-67 index is used as a proliferative marker and gives estimation of cells in a given tumor going through steps of active cell-cycle, hence, tumor size varies accordingly which is one of the components of TNM staging depicting prognosis of ductal breast carcinoma.[5] High Ki-67 is associated with prognosis of the breast cancer and is used routinely for treatment and selection of therapy.[5,8] Ki-67 levels are related to neoadjuvant chemotherapy because cells in division phase are highly sensitive to cytotoxic medications/ drugs[9]. An important issue in patient management is the response to ongoing treatment which is successfully guided by baseline Ki-67.[11,12] An effort to study the Ki-67 proliferative status, in form of index, as a validated marker for assessment of feed-back of ongoing chemotherapy is needed.[13]

Her-2 is an oncogene and a member of an epidermal growth receptor family having tyrosine-kinase activity.[13] The detection of amplification of this oncogene resulting in over-expression of Her-2/neu protein signifies its integral part in modulating landscape of invasive breast carcinoma.[6,11] The status of Her-2/neu receptor is required for the stratification of patients most likely to be benefited from targeted hormonal therapy i.e. epidermal growth factor receptor inhibitors.[5] In Trastuzumab therapy Her-2/neu receptor inhibitor has been related with good therapeutic response and improved prognosis in invasive ductal breast carcinomas having positive Her-2 protein expression and/or Her-2 gene amplification.[6,14] Over expressed protein of Her-2/neu receptor is being observed related to worsening grades of infiltrating ductal carcinomas (breast).[11]

Keeping in the above mentioned facts, present study was designed to see association between Ki-67 index and Her-2 receptor expression status in invasive ductal carcinoma (breast). The therapeutic strategies may further be upgraded as well as patient stratification may be refined with the establishment of such correlation of various biological modulators.[8]

Methodology:

In our hospital Ki-67 index is used as factor characterizing prognosis in patients of breast cancer. Data were collected from April 2019 to January 2020 from the Fatimah Jinnah Medical University, Lahore. Random 143 females having primary invasive ductal breast carcinomas from female patients of all age groups and exhibiting any of the grades were included by adopting convenient sampling process. Sample size was calculated by Cochran formula with Z score (Z) as 1.96 at 95% confidence interval, margin of error as .05 and expected prevalence (p) as 31%[2], however, due to certain constraints including training duration, 143 samples were included. The cases were selected after making diagnosis on routinely hematoxylin and eosin (H & E) stained sections made from Tru-Cut/core, wedge biopsies and mastectomy specimens[5]. The grading was assessed by consulting latest Bloom and Richardson's grading method modified by Elson and Ellis and for staging TNM system was consulted.[5,10]

.Ki-67 immunohistochemistry: Ki-67 expression was evaluated by immunohistochemistry performed on sections taken from tumor. Sections were cut from blocks prepared after tissue fixation in formalin then paraffin-embedding procedure.[8] Recommendations laid down by International Ki-67 in the Breast Cancer Workgroup provided basis for assessment of Ki-67 expression. According to these recommendations,

evaluation of Ki-67 labeling index is as a percentage of cells with positive nuclear staining after counting and analyzing 1000 cancer cells. Strong nuclear staining of Ki-67 having homogenous pattern in form of percentage of tumor cells up to 14 % were taken as high Ki-67 index while equal and below 14% was considered as low Ki-67 index.[5]

Her-2 immunohistochemistry: Her-2 expression status was evaluated by immunohistochemistry. Routine protocol of formalin tissue fixation and paraffin wax blocks making was followed to prepare sections of tumor tissue.[6] HER-2 receptor expression was scored using the scoring system in ASCO/CAP Protocols.[10] Her-2 score of 0 and +1 were considered as negative and +3 score was taken as positive for Her-2 protein expression.[5] Equivocal cases having score of +2 with HER-2 immunostaining were not included in study. Such equivocal cases require re-evaluation or confirmation through fluorescence in-situ hybridization (FISH) and facility of FISH was not available at institute where study was done.

Immunohistochemistry staining procedure was executed by me in supervision of an experienced colleague and interpretation and analysis was done according to International protocols.[18]

Data analysis was done on SPSS Statistics 20 version software (IBM Corp.,Chicago). Statistically significant cut off was taken with p-value of <0.05.

Results:

All 143 cases were invasive ductal carcinoma of the breast from female patients of age ranging from 32 to 58 years. From a total of 143 cases, 74 cases (51.4%) were with high Ki-67 index (>14%) while the remaining 69 cases (48.6%) were low proliferative index (<14%). Her 2 neu positive cases are 103 while negative cases are 40. In Table 1 Numerical data is presented as mean and range, however categorical data (age) is documented as frequency and percentage.

Table 2 shows the association between the Ki-67 index and Her-2/neu receptor expression by using Chi-Square test.

In Figure 1 correlation between Ki-67 and Her 2 expression is demonstrated.

Table 1: *Patients Characteristics (n=143).*

Patient Characteristics	n	%	Median	Min-Max
Age	143		45	32-58
Histological Grade	117	81.3		
2	26	18.1		
3				
Ki Expression %				
<14	69	48.9		
>14	74	51.4		
Her 2 Expression				
Positive	103	71.5		
Negative	40	28.5		

Table 2: *Correlation between Ki-67 Index and Her-2 Receptor Expression*

	Ki-67 Index <14	Ki-67 Index >14	Total
Her-2 Positive	22	18	40
Her-2 Negative	47	56	103
Total	69	74	143

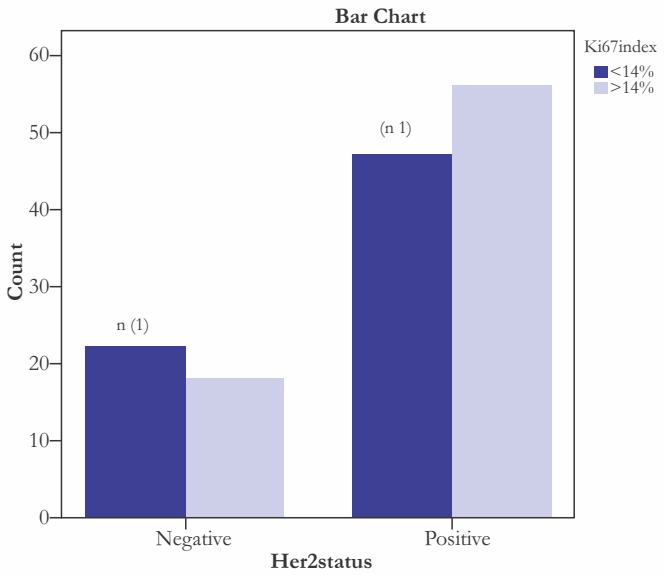

Figure 1: *Bar chart representing Ki-67 index and Her-2 receptor expression*

Discussion:

Breast carcinoma is the second commonest cause of mortality among females in Pakistan.[15] The modalities

are under continuous strive through diversified aspects to ameliorate prognosis in ductal breast carcinoma and making earliest categorization of patients for targeted therapy.[16]

In many studies higher tumor grade is significant for Poor Prognosis 19-24. During selection of therapy and to assess response of ongoing management, Ki-67 index act as most important decision marker.[13] The over-expression of Her-2 gene as revealed by enhanced receptor protein expression through immunohisto-chemistry is predominantly seen in infiltrating ductal carcinoma of breast as compared with other types[11].

In present study findings have been in concordance with the studies done by Yamanouchi K. 7 and Abir A. Muftah[16] in reproducing breast ductal carcinoma of infiltrative type showing higher index of Ki-67 also revealing positive Her-2/neu receptor protein overexpression. The same correlation has been observed by Ariga R. et al.[12] Correlation between 0 or +1 score of Her-2 expression and decreased proliferative rate of Ki-67 has been mentioned in one of the studies.[9] The current study also reveals that infiltrating ductal carcinomas of the breast with low Ki-67 index significantly have negative Her-2/neu protein expression status. This statistically highly significant association among Ki-67 index and Her-2/neu receptor protein status may be helpful in earlier stratification of patients for whom Her-2 inhibitors may be more beneficial based on proliferative index.[14] This correlation may prove to be fruitful in making treatment strategies in the integration of anti-cancer and hormonal therapies. Majority of breast carcinoma patients become unable to show desirable response from pathological point of view, after an integrated regimen. Hence, expression of receptors along with Ki-67 status are required to be reassessed in such patients of invasive ductal breast cancers that may also help in choice of therapy-related planning.[13] In order to achieve complete clinical utilization of Ki-67 and to get maximum benefits of its potential role, an elaborated and variable modulations in methodology, improvement in scoring system and cutoff points, advancement in standardization and laboratory accreditation is essential, as have already been established with estrogen, progesterone and HER2 receptors.

As both factors, Ki-67 index and Her-2/neu receptor protein have independent predictive, prognostic and therapeutic significance so the association between these markers can be helpful in stratification of patients according to the risk of recurrence and also in separating cases having probability of exhibiting resistance to treatment and in making improved treatment strategies for cases having aggressive behavior.[12,21] It may prove to be a helpful ancillary tool in evaluating the behavior in equivocal cases of HER-2/neu immunohistochemistry, that show score 2+ and are required to be evaluated by FISH for further confirmation[5].

Conclusion:

Our study conclusion is that highly significant correlation has been observed between higher index of Ki-67 and positive Her-2 receptor expression on immunohistochemistry. However Randomized control trials are required for improved and elaborated comparison of these prognostic factors especially with more number of cases according to the calculated sample size.

Ethical Approval: Given

Conflict of Interest: The authors declare no conflict of interest.

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