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Correlation of proliferative index with various clinicopathologic prognostic parameters in breast carcinoma

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Abstract---Introduction: Breast cancer (BC) is the most common cancer and the second most common cause of death in women. Prognostic factors are essential in BC diagnosis as they allow the identification of high-risk patients, for whom, an adjuvant therapy can improve prognosis. Interest in Ki-67 has recently increased as Ki-67 is a potential marker for predicting the responsiveness to chemotherapy. Materials and Methods: The study was conducted on 75 consecutive cases of primary breast carcinoma undergoing radical or modified radical mastectomy specimen. Histopathological diagnosis was established on routine hematoxylin and eosin stain and various histologic prognostic parameters including histologic type, histologic grade, and lymph node metastases were assessed. All biopsy proven carcinoma breast were included in this study. Results: Among presenting compliant, 47 (62.66%) had lump in right breast and 28 (37.33%) had lump in left breast. Most patients in the study population presented with a lump of duration 3-6 months (54.6%). Out of 75 participants, 41 (62.66%) participants were diagnosed with carcinoma right beast and 28 (37.33%) with carcinoma left breast. On staging the disease among the study population, most of them diagnosed with breast cancer were of stage IIIB (48%) followed by IIA

(25.33%). Conclusions: Globally, breast cancer is the most common cancer in terms of incidence and mortality in women, which raises a social problem and a threat to the women health community. It is an appropriate method to use in prognosis determination and the selection of proper therapy protocol. In other words, the correlation between the prognostic factor, Ki-67, and other prognostic and predictive factors assure the physicians a better management in patients with BC.

Keywords---proliferative index, breast carcinoma, Ki-67.

Introduction

Breast cancer (BC) is the most common cancer and the second most common cause of death in women. Globally, one in 14 women will develop breast cancer between the age of 0-79, which comes down to 1 in 9 women in the developed countries. [1] Breast cancer is the most common cancer among Iranian women and ranked the 5th leading cause of cancer death in women. [2] Prognostic factors are essential in BC diagnosis as they allow the identification of high-risk patients, for whom, an adjuvant therapy can improve prognosis. [3] The traditional prognosis can only identify the group of approximately 30% of patients and their outcome. Therefore, new prognostic markers are required. [5] Considering the fact that radiotherapy and different medical hormonal manipulations may develop side effects in patients, the risk-based refined approaches are essential to reduce these side effects. Some new prognostic factors have been described over the last few years. [5] However, most of them still require clinical validation. [6]

In exploration for the potential prognostic indicators of breast cancer, attention has been focused on tumor markers. Cell proliferation plays an important role in the clinical behavior of invasive BC. Increased cell proliferation is correlated with poor prognosis. [7] Ki-67 labeling index is more sensitive than other techniques such as mitotic figure counts because cells with active phases of the cell cycle can be recognized; furthermore, a reliable assessment of mitotic figures is more time-consuming than the counting of nuclei in immunohistochemistry (IHC). The reproducible mitotic index is not usually obtained without special training in counting with fraction assessed method, yet mitotic count and Ki-67 index are still considered to be the most practical methods. [8]. Interest in Ki-67 has recently increased as Ki-67 is a potential marker for predicting the responsiveness to chemotherapy. [9] Compared with other markers, Ki-67 immuno-staining is a convenient method for assessing the proliferating index. Ki-67 IHC is a rapid and inexpensive technique that can be easily used in almost all pathological laboratories and requires only a small tissue sample, including those obtained from fine-needle aspirations. Ki-67 levels are known to be associated with positive prognosis in most studies. [10]

Materials and Methods

The study was conducted on 75 consecutive cases of primary breast carcinoma undergoing radical or modified radical mastectomy specimen. Histopathological

diagnosis was established on routine hematoxylin and eosin stain and various histologic prognostic parameters including histologic type, histologic grade, and lymph node metastases were assessed.

Inclusion Criteria

All biopsy proven carcinoma breast were included in this study

Exclusion criteria

- Patients who are treated with neoadjuvant chemotherapy.
- Male carcinoma breast patients.
- Recurrent carcinoma breast patients.
- Patients with distant metastasis.

Immunohistochemistry

Tissue sections (3–4 μ) from formalin-fixed, paraffin embedded block of each case were taken on poly L-lysine coated slides. After deparaffinization in xylene and rehydration through graded alcohol, antigen retrieval was done in Tris ethylenediaminetetraacetic acid in pressure cooker. Endogenous peroxidase activity was blocked by using peroxidase block for 20 min followed by protein block for 15 min. Thereafter, the slides were incubated with one of the following primary antibodies: Anti-human ER, PR, HER2/neu, and Ki-67. Following treatment with secondary antibody for 30 min and 3,3'-diaminobenzidine tetrahydrochloride chromogen for 5 min, the slides were counterstained with Harris hematoxlylin. Appropriate positive and negative controls were run with each IHC batch.

Ki-67 expression

Dark brown diffuse or grainy nuclear staining was taken as positive. Ki-67 labeling index (Ki-67-LI) was estimated as the number of positive nuclei divided by total number of nuclei scanned counting a minimum of 1000 cells in 10 selected high-power fields that displayed highest immunoreactivity and was expressed as percentage. Tumors were classified as positive where >5% of cells expressed positive staining10 and as highly positive if >20% of tumor cells expressed Ki-67. [11] We had selected a value of 5% as positive since it represented a figure which is in excess of that determined for normal and benign breast tissue as also described by Bouzubar *et al.* [12] A cut-off of 20% chosen by us as a criterion of high positive was within the range of cut-offs from earlier published studies (5–30%), defined as optimized cut points, median cut points, or categorized into three groups. [13]

Statistical analysis

The results obtained were interpreted and correlated statistically. Mean and standard deviations were calculated. When the data was qualitative, a Chi-square test was used to assess the association between these parameters. A value of $P <$

0.05 was taken as significant (S) and <0.01 was taken as highly significant (HS) whereas the $P > 0.05$ was taken as nonsignificant.

Results

Table 1
Descriptive Analysis of Age (In Years) in Study Population (N=75)

Age	Number Of Patients	Percentage(%)
<30 Years	2	2.66
31-45 Years	19	25.33
46-60 Years	37	49.33
>61 Years	17	22.66

Table 2
Descriptive Analysis of Presenting Complaint in the Study Population (N=75)

Presenting Complaint	Frequency	Percentages
Lump in right breast	47	62.66%
Lump in left breast	28	37.33%

Among presenting compliant, 47 (62.66%) had lump in right breast and 28 (37.33%) had lump in left breast. (Table 2)

Table 3
Descriptive Analysis of Duration of Symptoms in Study Population (N=75)

Duration of Lump	Frequency
0 - 3 months	25
3 - 6 months	41
>6 months	9

Most patients in the study population presented with a lump of duration 3-6 months (54.6%).The mean duration of lump was 4.93 ± 0.58 months (Table 3)

Table 4
Descriptive Analysis of Diagnosis in The Study Population (N=75)

Diagnosis	Frequency	Percentages
Carcinoma Right Breast	47	62.66%
Carcinoma Left Breast	28	37.33%

Out of 75 participants, 41 (62.66%) participants were diagnosed with carcinoma right beast and 28 (37.33%) with carcinoma left breast. (Table 4)

Table 5
Descriptive Analysis of Staging in The Study Population (N=75)

Staging	Frequency	Percentages
I	0	0%
IIA	19	25.33%
IIB	11	14.66%
IIIA	9	12.0%
IIIB	36	48.0%
IV	0	0%

On staging the disease among the study population, most of them diagnosed with breast cancer were of stage IIIB (48%) followed by IIA (25.33%) (Table 5).

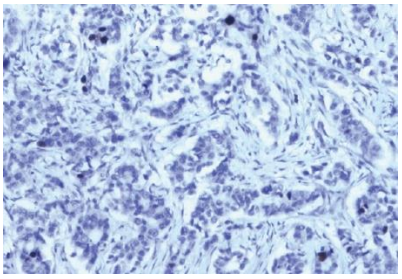

Figure 1. Ki-67 expression in Grade I Invasive ductal carcinoma

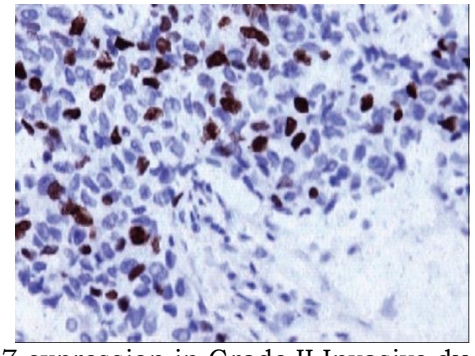

Figure 2. Ki-67 expression in Grade II Invasive ductal carcinoma

Table 6
Descriptive Analysis of Investigation Findings in The Study Population (N=75)

Investigation findings	Frequency	Percentages
Histopathology		
Infiltrating ductal carcinoma	75	100.00%
Estrogen receptor status		
Positive	39	52.00%
Negative	36	48.00%
Progesterone receptor status		
Positive	35	46.66%
Negative	40	53.33%

Her2Neu status		
Equivocal	2	2.66%
Positive	26	34.66%
Negative	47	62.66%

Among the investigation findings, all of them had infiltrating ductal carcinoma, 39 (52.00%) had estrogen receptor positive status, 35 (46.66%) had progesterone receptor positive status and 2 (2.66%) had equivocal expression of hormone receptor status and 36 (36.73%) had her2neu positivity (Table 6)

Table 7
Descriptive Analysis of Ki 67 Classification in Study Population (N=75)

Ki 67 Classification	Frequency	Percentages
<20	29	38.66%
≥20	46	61.33%

Among the study population, 46 (61.33%) had ki67 proliferation index ≥20 and 29 (36.66%) had<20. (Table 7)

Table 8
Comparison of Mean Size of Lump with Ki 67 Index (N=75)

Parameter	KI 67 classification (Mean± SD)		P value
	<20 (N=29)	≥20 (N=46)	
Length (in cm)	5.01 ± 0.62	4.24 ± 0.58	0.525
Width (in cm)	4.03 ± 0.53	3.83 ± 0.47	0.375

The mean <20 ki67 classification in size of length was 5.01 ± 0.62 cm and the ≥20 ki 67 in length was 4.24 ± 0.58 cm, the association between two groups was statistically not significant (P value 0.525). The mean <20 ki67 classification in size of width was 4.03 ± 0.53 cm and the ≥20 ki67 in length was 3.83 ± 0.47 cm, the association between two groups was statistically not significant (P value 0.375). (Table 8)

Table 9
Comparison of Staging with Ki 67 Index (N=75)

Staging	Ki 67 Classification		Chi square	P value
	<20	≥20		
Stage I	0 (0%)	0 (0%)	2.754	0.371
IIA (N=19)	9 (47.36%)	10 (52.63%)		
IIB (N=11)	2 (18.18%)	9 (81.81%)		
IIIA (N=9)	3 (33.33%)	6 (66.66%)		
IIIB (N=36)	15 (41.6%)	21 (58.33%)		
Stage IV	0 (0%)	0 (0%)		

Stage IIIB (36) was the most prevalent stage among the subjects considered in the study. Among them 30 had a ki67 index ≥20 which was not statistically significant. (Table 9)

Discussion

This Prospective study investigated the value of Ki67 as a predictive factor in relation to neoadjuvant chemotherapy and possible effects on prognosis. Ki67 was found to be an independent predictor for pathological complete responses and for the prognosis in all patients across all subtypes. Although a number of new methods have been developed, Ki-67 antigen has been widely used and until the reliability of these new methods is confirmed, the current standard proliferation assay should be Ki-67 IHC, given its relative simplicity and wide availability. [13] Moreover, the intratumoral heterogenecity of Ki-67 is insufficient to mask its representation of proliferation. [14] Ki-67-LI has been expressed as the percentage of the total number of tumor cells that stain positive which equates to the growth fraction of the tumor. [15] Although there is no international standardization for the method, however, Ki-67 and mitotic frequency have just recently been included in the St. Gallen guidelines which have accepted the cut-off value of Ki-67-LI as 15%, but it is noted that the reliability of these measures still varies in different geographic settings. [16] However, a study comparing the proliferation rate of breast cancer patients between Asian and Western populations has defied this variation. [17]

Bouzubar *et al.* considered a value of <5% as negative, while Tan *et al.* nil staining as negative. Bouzubar *et al.* and Tan *et al.* defined high Ki-67-LI as >20% and >10% respectively. [18] Zhang *et al.* used Ki-67 to separate the patients into two prognostic groups, cases with >15% Ki-67-LI belonging to the poor prognostic group. [19] Cho *et al.* suggested that the 20% cut-off value be the preferable value in clinical practice. [20] In our study, Ki-67 was assessed in form of Ki-67-LI, defined as percentage of positive tumor cells and Ki-67-LI of <5%, 5–20%, and >20% were taken as negative, positive, and high positive, respectively. In the study, Ki-67 expression was correlated with various clinicopathologic parameters including menopausal status, tumor size, lymph node status, histologic grade, NPI, and hormone expression. No significant association was seen between Ki-67 expression and menopausal status in our study and other studies as well. [21]

A direct statistically significant direct correlation was found between Ki-67 expression and tumor. Similar observations were made by Querzoli *et al.* [22] They also categorized their cases in three sizes <2 cm, 2–5 cm and >5 cm and found a direct correlation between size and Ki-67-LI. Tumor size, however, is not consistently linked to Ki-67 score with some authors finding a positive relationship [23] but others did not. [24]. Lymph node status has a controversial association with Ki-67-LI. In our study, the comparison of Ki-67 with this important prognostic variable did not reveal any major association and our result complements various studies in literature, [25] but is at slight variance with the data of Lellé *et al.* [26] and Querzoli *et al.* [22] who suggested significant association of high Ki-67-LI with lymph node metastasis.

The Ki-67 expression had a significant correlation with histologic grade of the tumor. Though each grade category contained both low and high Ki-67-LI cases, most of Grade III tumors revealed high positivity. Of the commonly used pathological features of breast cancer, the histologic grade is probably the most robustly related to Ki-67 with virtually no study refuting this positive correlation. [27] This is to be expected given that mitotic index is one of the three components of grade. [28] Zhang *et al.* in their study further analyzed the role of Ki-67-LI to provide additional prognostic information in grading, especially for patients with Grade II tumors. [19] Histologic Grade II, a group with intermediate and variable prognosis, constitutes a problem when selecting adjuvant medical treatment and by stratifying Ki-67, this group can be divided into two groups with significant difference in prognosis of patients with histologic Grades I and III, especially in node negative cases. [29]

When Ki-67-LI was correlated with NPI, the association was found to be statistically significant. Although the size, tumor grade, and lymph node status have been independently correlated with Ki-67 expression in previous studies, none of these studies has evaluated the relation between Ki-67 and NPI. [30]. The accuracy and reliability in grading have always been a matter of concern, hence, the reproducibility of grading should be enhanced. Ki-67-a proliferation marker is easily identified and provides comparable accurate information. In contrast to poor reproducibility of mitotic counts, Ki-67 can achieve high agreement between pathologists; is more reproducible; adds complementary value to the MBR grading system and correlates well with other clinicopathologic parameters. It may act as a significant prognostic indicator for routine clinical use and be helpful for selection of adjuvant treatment. It can also add value in categorizing Grade II tumors into two prognostic subgroups with prognosis equivalent to Grades I and III, respectively.

Conclusions

Globally, breast cancer is the most common cancer in terms of incidence and mortality in women, which raises a social problem and a threat to the women health community. The recent molecular biology research provides useful information for personalized treatments. Therefore, patients will have more chance to choose a suitable therapy, which can prevent complications. Inaccessibility to molecular biology techniques among the communities with financial difficulty has led to the limited exploitation of these methods in treatment. It is an appropriate method to use in prognosis determination and the selection of proper therapy protocol. In other words, the correlation between the prognostic factor, Ki-67, and other prognostic and predictive factors assure the physicians a better management in patients with BC.

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