RESEARCH ARTICLE

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Proliferative Index (Ki67) for Prediction in Breast Duct Carcinomas

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Abstract

Background and objectives: To date, many tumor markers have been used to predict prognosis and therapeutic response in patients with breast cancer. The well established and routinely applied tumor markers are the estrogen-receptor, progesterone-receptor and Her2/neu-receptor. In the current study, we aimed to highlight any association of the proliferation index (Ki67) in breast infiltrative duct carcinoma with the tumor grade, tumor size and nodal status in addition to hormone receptor status. Tissue sections were stained immunohistochemically for Ki67 nuclear antigen, estrogen, progesterone and Her2/neu receptors using an automated Dako machine (Dako Denmark. There was a significant inverse relationship of Ki67 levels with ER and PR, while values were directly proportional to the tumor grade and Her2/neu status. No significant association was found between Ki67 and size of tumor or nodal status. Ki67 immunoexpression may offer an independent predictive tumor marker and for routine application in cases of breast cancer.

Keywords: Breast cancer- Ki67- immunohistochemistry

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Introduction

Globally, breast cancer (BC), mainly the infiltrative duct carcinoma (IDC), is the most frequent women's malignancy, accounting for about 25% of all cancers and is the leading cause of cancer death among women(international Agency for research on cancer, 2012; America cancer society, 2012). By the latest copy of the Iraqi cancer registry, breast cancer ranks the first of the top ten cancers in females, and about 23 per 100,000 female populations develop breast cancer (Iraqi cancer registry, 2011). However, the overall breast cancer death rates declined in the last 10 years owing to the improvements in early detection and treatment approaches(Esteva and Hortobagyi, 2004). Advances in diagnostic methods recommend the use of tumor markers for predicting the prognosis and therapeutic guidance that positively influence tumor progression. The most established tumor markers comprise estrogen receptors (ER), progesterone receptors (PR) and Her-2/neu receptors (Esteva and Hortobagyi, 2004).The Ki67, a marker of cell proliferation, is a non-histone nuclear protein expressed throughout the active phase of cell cycle, except G0 and early G1. This proliferative marker has become an important and reliable predictive and prognostic marker in BC patients (Scholzen and Gerdes, 2000; Esteva and Hortobagyi, 2004). The threshold value for defining high and low expression of Ki67 labeling index is still a matter of debate and not yet standardized. In the current study, we used 14% as a threshold value for Ki67 labeling index depending on what has been reported by many studies where the staining level of ≥14% as high risk group in term of prognosis (Cheang et al., 2009; Kristina et al., 2013; Wang et al., 2016).

Materials and Methods

One hundred and twenty six patients undergoing mastectomy or lumpectomy for IDC of breast during a 41 month-period, from January/2014 to June/2017, were enrolled in the study. Paraffin blocks in addition to information related to patient age and tumor size were taken from histologic reports retrieved from pathology department at Vin Private Medical Laboratory, Duhok-Iraq. Tissue sections were stained again with H and E for cancer grading and lymph node status. The four-tired "Bloom-Richardson" standard grading system was applied depending on the nuclear pleomorphism, tubular formation, and number of mitoses (Kumar et al., 2013).

Unstained tumor sections were subjected to immunohistochemical stains according to the manufacturer's instructions (Dako Denmark, A/S), using the autostainer (Dako Denmark, Link48), for estrogen receptors (ER: Era EP1) and progesterone receptors (PR:

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PgR, 636). More than 5% of tumor cells with brown stained nuclei for ER and PR were considered positive, cytoplasmic stained cells were ignored. Her-2/neu (c-erbB-2) receptor positivity and Ki67 status were done as described previously(George et al., 2015) For Her2/neu sections, Hercept test-kit was applied to evaluate the results as follows: 0 or +1 (negative) when there was no or faint staining of tumor cell membrane respectively; +2 (borderline) when >10% of tumor cells showed a weak to moderate membranous staining; and +3 (strong positive) when >10% of tumor cells showed a strong and complete brown stained membrane (Pity and Jalal, 2013; Wolff et al.,2013). Assessment of Ki67 (MIB1) expression was estimated as the percentage of positively stained tumor cell-nuclei. Cases with more than 14% stained nuclei were considered as high proliferative index while those with equal or less than 14% positive nuclei were reported as low (Cheang et al., 2009; Kristina et al., 2013; Wang et al., 2016).

Appropriate positive controls were processed parallel with each set of IHC technique, using internal non-neoplastic breast acini for ER and PR; Her2-strongly positive breast cancer for Her2; and a lymph node with Burkitt's lymphoma for Ki67. Negative controls were accomplished by incubating non-stained sections with buffer solution instead of the primary antibodies.

Classical SPSS version 16 was used in all statistical tests (SPSS Inc, Chicago, IL, USA), t-test and ANOVA with pairwise comparison test were used for comparing variables of different categories. Duncan correlation was used for analysis of differences between groups. Defining the association between clinical parameters was done using Two-tailed Pearson Chi-square test. The p value of ≤ 0.05 was considered as significant.

Results

This study explored the pathological specimens of 126 women with IDC, aged 23-75 years (mean 49.7 yr.). The results of staining were categorized into four groups; ER -/PR-ve,HER2/neu+ve;Triplenegative(ER-/PR-/Her2/neu-);ER+/PR+/Her2/neu-and the last group Triple positive(ER+/PR+/Her2/neu+).Twenty-two (17.46%) cases were HER2/neu positive and ER/PR negative .Triple negativity was observed in 14 (11.12%) cases. HER2/neu negativity with ER/PR positivity was found in 54 (42.85%) cases. The remaining 36 (28.57) cases were triple. The proliferative index (Ki67 expression) was high (> 14 %) in 110 (87.3%) specimens and low (≤ 14%) in the remaining 16 (12.7%) cases (Table 1). Photographs for positive ER, PR in addition to negative and positive Her2 were illustrated in Figures 1-4, and different values of Ki67 status were shown in Figure 5.

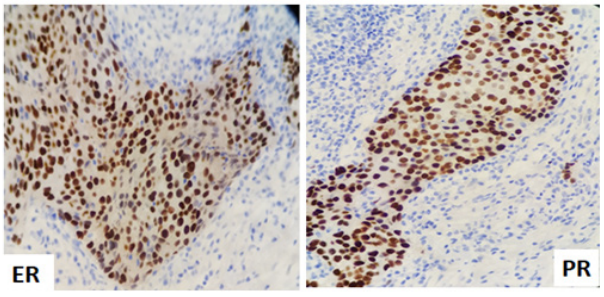

Figure 1. A Strong Nuclear Positivity for Estrogen Receptors "ER" and Progesterone Receptors "PR" (IHC, x400).

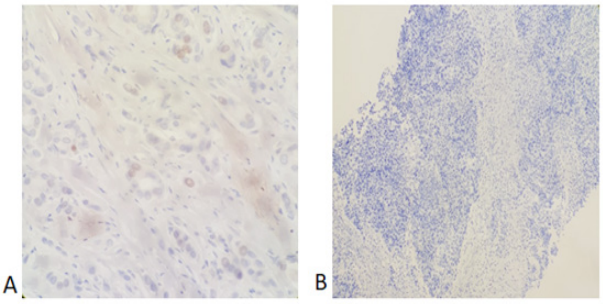

Figure 2. Negative Her2; +1 in A and Completely Absent in B (IHC, x200).

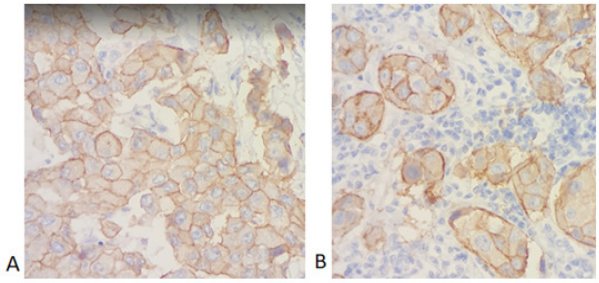

Figure 3. Borderline Positivity for Her2/neu "+2" (IHC, x400).

There was a significant inverse relationship of the proliferative index (Ki67) with ER (-0.216, p= 0.015) and PR (-0.182, p= 0.041). In contrary, Ki67 was found to be directly proportional to the Her2/neu status (0.309, p= 0.001,). On the other hand, no any significant association was found between the proliferative index Ki67 neither with the size of the tumor nor with the number of lymph node involved (N) (Table 2).

As shown in Table 3 below, there was a significant association between Ki67 expression and cancer grades with the lowest index among grade I and highest among grade III cancers (p= 0.001).

Regarding the hormonal status categories, the "HER2/

Table 1. Characterization of Different Hormone Receptors Groups According to the Results of Immunoexpression of ER, PR, Her2/neu and the Number and Percentage of Cases with Ki67 Expression Above and Below the Cutoff Point (14%) in the Study Cases.

Variable	No. of cases	Percentage
ER-, Pr-, HER2/neu +	22	17.46
Triple negative*	14	11.12
ER+, PR+, Her2/neu -	54	42.85
Triple positive **	36	28.57
High Ki67 (> 14%)	110	87.3
Low Ki67 (≤ 14%)	16	12.7

*ER-/PR-/HER2; **ER+/PR+/HER2+

Table 2. Relation of KI67 Expression with Different Parameters in BC Cases

	Age	Size (cm)	N	ER	PR	Her2	Ki67	Grade
Size (cm)	0.093							
	0.298							
N	0.071	0.172						
	0.432	0.054						
ER	0.084	-0.082	0.06					
	0.352	0.359	0.503					
PR	-0.045	-0.097	0.021	0.687				
	0.615	0.282	0.815	0				
Her2	0.02	-0.046	0.099	-0.201	0.204			
	0.828	0.606	0.272	0.024	0.022			
Ki67	-0.081	0.098	0.116	-0.216	-0.182	0.309		
	0.369	0.275	0.197	0.015	0.041	0		
Grade	0	0.132	0.29	-0.174	-0.13	0.41	0.432	
	0.998	0.139	0.001	0.052	0.148	0	0	
Type	-0.024	-0.058	-0.001	0.025	0.043	-0.097	0.09	0.09
	0.787	0.521	0.993	0.778	0.631	0.282	0.316	0.316

ANOVA with pairwise comparison test. The upper numbers in each space represent the type of relationship between Ki67 and the parameter above. If negative means "inverse relationship," if no sign means "direct relationship" while the lower numbers represent the "p" value)

Table 3. Association of Ki67 Expression with Cancer Grade

	Cases	Ki67	
Grade	No. (%)	Mean ± SD	P
I	11 (8.7)	19.45±14.63	0.001
II	54(42.85)	35.89±18.99	(Significant)
III	61(48.41)	50.79±24.24	
IV	0 (0.0)		

P value, 0.001 (ANOVA test).

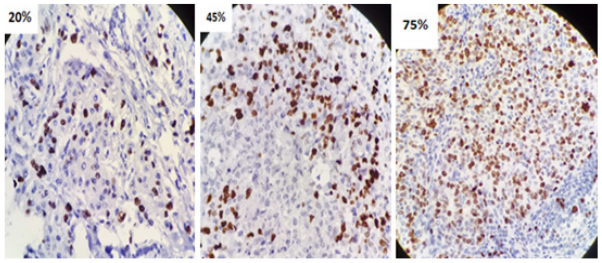

Figure 5. Representative Images of High Ki67 Immunoexpression.(IHC, x200).

neu+/ER-/PR-" and the triple negative "HER2/neu-/ER-/PR-" groups expressed a significantly high Ki67 expression (55.50±4.45; 46.69±6.76 respectively). However, no any significant relationship was observed between these two groups regarding Ki67 expression. In contrast, Her2-/ER+/PR+ cancer cases were found to be significantly associated with low Ki67 expression (32.74±2.83) with a significant difference from the above two groups, but not the triple positive group (Table 4).

As well, a significant relationship was demonstrated between high Ki67 level and Her2/neu positivity (Table 5). The mean Ki67 was higher (52.50±22.3) among women with positive HER2/neu, while lower means of Ki67 were among women with negative and borderline HER2/neu, reflecting a raised proliferative index with the increase of Her2/neu positivity status.

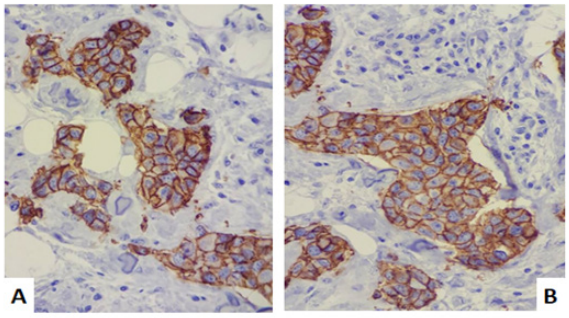

Figure 4. Strong Her2/neu Positivity "+3" (IHC, x400).

Discussion

Among our 126 cases of ductal carcinoma, 17.46%

Table 4. Ki67 Expression in Different Receptor Categories

Variable		Ki67		
	N	Mean ± SE	Minimum	Maximum
ER-/PR-/Her2/neu+ve	22	b (55.50±4.45)	20	95
Triple -ve	14	b (46.69±6.76)	15	87
ER+/PR+/Her2/neu-	54	a (32.74±2.83)	1	80
Triple positive	36	a, b (43.40±3.87)	4	90
Total	126	(41.25±2.08)	1	95

*The letters "a" and "b" indicate significant difference between different categories (Duncan test). Similarity between the letters of groups means no difference between them while different letters reflect difference between the groups.

Table 5. Difference in Ki67 Expression in Correlation with Her2/neu Status

	Cases	Ki67	
HER2/neu	No. (%)	Mean ± SD	P
Negative	68 (54%)	36.32±22.86	
Borderline	19 (15%)	41.7±23.40	(Significant)
Positive	39 (31%)	52.50±22.30	
Total	126		

ANOVA test used.

of cases were HER2/neu+/ER-/PR-, 42.85% were HER2/neu-/ER+/PR+ and 11.12% represented the triple negative group. The triple positive category formed the remaining 28.57%. These findings were close to Onitilo et al study(2009) in USA who reported 17.7% HER2/neu+/ER-/PR, and 13.4 % triple negative and agree with Effi et al results (2017) in Ivory Coast women, who found 43% of BC cases were ER/PR positive.

No optimal cutoff point for Ki67 proliferative index has been standardized yet and this may be responsible for the difficulty in choosing a standard threshold for daily practice. In the current study we chose 14% Ki67 expression value to classify breast cancer cases into low risk category (<14%) and high risk category (>14%) depending on what have reported by other studies (Cheang et al., 2009; Fasching et al., 2011; Kristina et al., 2013; Wang et al., 2016).

In this study, 87.3% of cases presented with positive Ki67 (>14%). This finding is similar to that of a previous study conducted in India by Kaur et al., (2016). A fact that empower the concept stated Ki-67 is a significant marker considering the proliferative capability of breast cancer cells (Luporsi et al., 2012; Tawfik et al., 2013).

We fail to demonstrate any significant relationship between Ki67 expression neither with tumor size nor with number of lymph nodes involved by metastasis. Here we may say that Ki67 may act as independent prognostic factor, just in contrast to what has been reported by Li et al., (2014) among Chinese women, who postulated that the prognostic value of Ki67 is predicted by the number of positive lymph nodes number. Similarly, Matsubara (2011) and his colleagues in their study among Japanese women used 10% Ki67 cut off point and denied any impact of Ki-67 overexpression as an independent prognostic factor for overall survival. Other studies found a significant relationship of affected axillary lymph nodes and >14% Ki67 positive cells in BC women especially those with the worst prognosis (Nishimura et al., 2010; Haroon et al., 2013; Gonzalez et al., 2014; Martins et al., 2015; Yan et al., 2015).The fact behind this disagreement could be explained by the differences in the Ki67 cut off point. (Nishimura et al., 2010; Matsubara et al., 2011; Haroon et al., 2013), the organ studied (Martins et al., 2015), the considerable intra tumoral heterogeneity of Ki67 expression (Ruiz et al., 2006), as well as the limited sample size used in our study compared with others, beside the fact that we excluded many of our cases as they were missing hormone status measurement.

Considering each biomarker individually, our results showed a significant inverse relationship of Ki67 with ER and PR in contrast to the direct proportion with Her2/neu status. Different associations were demonstrated in the literatures (Ruiz et al., 2006; Yan et al., 2015). However, Haroon et al., (2013) reported in their study among Pakistanian women with BC, a positive association of Ki67 with PR and Her2/neu but not with ER. On the other hand Sheikhpour and Poorhosseiniz (2016) who found a reverse correlation between ER and PR with Her2/neu in their study among Iranian women, denied any meaningful relation of ER/PR with Ki67 immunoexpression .

Giving different (Her2/ER/PR) categories of BC, we observed differences in Ki67 expression with a significant decline in Ki67 expression in sections with HER2/neu –ER+/PR+ compared with Her2+/ER-/PR- and triple negative groups, but not different from the triple positive group. Similarly, Haroon et al., (2013) study in Pakistan reported a higher correlation between positive ER/ PR and Ki67 expression.

The significant direct relationship between Ki67 expression and HER2/neu positivity among our series is similar to what have been reported by many authors who detected a higher correlation between HER2/neu and increased expression of Ki67 among breast cancer women (Ruiz et al., 2006; Zaletok et al., 2012; Haroon et al., 2013; Inwald et al., 2013; Nishimura et al., 2014; Shokouh et al., 2015; Yan et al., 2015; Sheikhpour and Poorhosseini, 2016).

Furthermore, our study showed a significant association high Ki67 expression and grade-III cancers, suggesting that higher proliferative index reflects hyper-proliferation, poor tumor differentiation and thus worse prognosis. This finding coincides with results of the Elkablawy et al., (2016) study in Saudi Arabia and Madani et al., (2016) study in Iran who documented that Ki67 over expression is significantly related to high grade BC. In same line, other literature explored the great association between tumor grading and Ki67 index, and thus its prognostic role in BC women (Trihia et al., 2003; Inwald et al., 2013; Sheikhpour and Poorhosseini, 2016).

The main limitations of the present study can be declared by the fact that it was a single center study with missing some histopathological parameters, particularly distant metastasis (M) stage-status in addition to the relatively low Ki67 cut-off value used.

In conclusion, this study extended what has been reported previously that besides ER, PR and HER2, the proliferative marker (Ki67) can be used in BC as an indicator to obtain prognostic information and may help for therapeutic decisions.

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