JOURNAL OF INTERDISCIPLINARY HISTOPATHOLOGY, 2018
VOL 6, NO. 1, PAGE 1–10
10.5455/jihp.20170331011238

eJManager

ORIGINAL RESEARCH

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Comprehensive analysis of the association of clinically relevant values of Ki-67 labeling index with clinicopathologic and immunohistochemical criteria in female invasive breast carcinoma

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ABSTRACT

Objective: Breast cancer aggressiveness is related to tumor cell proliferation. Despite this, the Ki-67 index is not recommended for routine use in newly diagnosed breast carcinomas.

Material and Methods: A total of 164 invasive breast carcinomas were stratified into the intrinsic molecular subtypes based on estrogen receptor, progesterone receptor (PR), HER2, and Ki-67 immunostaining. We studied the distribution of Ki-67 among the molecular subtypes and correlated it with clinicopathologic parameters. Furthermore, the change in the Ki-67 index with tumor size, grade and lymph node (LN) status among the molecular subtypes was examined.

Results: As a continuous variable, the median Ki-67 did not show significant differences with the clinicopathological variables. At a cutoff ≥ 14%, it correlated significantly with the mitotic index. At a cutoff ≥ 20%, it additionally correlated with the PR status. The median Ki-67 level varied significantly between luminal A and all other molecular subtypes. The median Ki-67 level in T1/T2 tumors compared to T3/T4 tumors was slightly higher in luminal B HER2+, slightly lower in HER2 enriched, and nearly similar among luminal A, triple negative and luminal B HER2-subtypes, yet without statistical significance. The median Ki-67 was lower in G1/G2 compared to G3 tumors in all-except luminal B HER2-positive subtype but without statistical significance. The Ki-67 distribution change between N0/N1 and N2/N3 cases among the molecular subtypes was significant.

Conclusions: The impact of Ki-67 as a proliferation marker on the biological behavior of breast carcinomas is context dependent, and its clinical utility increases when interpreted in combination with other prognostic markers in the context of the molecular subtypes. Further studies, on larger sample sizes are recommended to unravel how the molecular types can affect the relation between Ki-67 and clinicopathological characteristics, particularly the LN status.

ARTICLE HISTORY

Received March 26, 2018
Accepted March 06, 2017
Published April 22, 2017

KEYWORDS

Breast carcinoma, interaction, Ki-67, molecular subtypes, tumor biology

Introduction

Breast cancer (BC) is the most common female malignancy and the leading cause of female cancer-related deaths both globally (13.7%) and in Egypt (29.1%) [1]. Developing countries, in opposition to developed countries, experience an increase in the incidence of BC [2] with a notable increase in the biologically aggressive subtypes [2,3].

Because of a higher incidence of poor prognostic factors, late diagnosis and inadequate treatment regimens [4], the incidence to mortality ratio of BC in Egypt (3.7:1) compared to the global ratio (1.9:1) is poor [1]. Although adjuvant systemic therapy has contributed to decreasing BC mortality [5], yet it failed to prevent recurrence in a subset of high-risk hormone receptor (HR)-positive tumors [6].

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Multi-gene tests revealed that tumor proliferation is a significant predictor of the risk of tumor recurrence [7,8] and subsequently, the tumor proliferation fraction has become an established predictive marker of clinical outcome of BC patients [9-12]. A variety of techniques are available to assess the rate of tumor cell proliferation among which is the immunohistochemical (IHC) assessment of nuclear proliferation antigens [12,13].

Ki-67, a nuclear proliferation protein that regulates the cell cycle [10-12], is already part of multi-gene tests. It has been established as a reliable method for assessment of BC cell proliferation [14] to allow differentiation between luminal A and luminal B HER2-negative tumors as recommended by the St. Gallen Consensus Conferences; 2011 and 2013 [15,16].

Despite the fact that high Ki-67 index not only predicts a worse outcome in early BC [17-20] but also predicts the relative responsiveness to adjuvant systemic therapy [12], yet its inclusion in clinical decision-making is still debatable [17], because of the lack of standardization regarding its measurement [12]. Moreover, consensus has not been reached as regards the Ki-67 cutoff values for using chemotherapy and a grey zone still exists especially for intermediate Ki-67 levels regarding initiating adjuvant therapy based on the proliferation index for these intermediate Ki-67 levels [15,21]. Therefore, assessing the association of Ki-67 expression with other prognostic biomarkers including HR and HER2 status expression might prove helpful in clinical decision-making.

The aim of this study was to study the expression of Ki-67 among the different molecular subtypes, and to correlate the Ki-67 labeling index with the clinicopathological and prognostic biomarkers. Another aim was to study the interaction effect between the molecular subtype and some clinicopathological factors (tumor size, lymph node [LN] status, and tumor grade) on the distribution of Ki-67. In other words, we aimed to examine more closely the change in the distribution of Ki-67 with tumor size, LN status, and histological tumor grade to observe whether this change is the same among the different molecular subtypes or not.

Material and Methods

A total of 164 breast biopsy specimens of female invasive mammary carcinomas submitted to our Pathology Department, during the period from January 2012 to December 2014 were enrolled in this retrospective study. The cases were stratified into the intrinsic molecular subtypes based on estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 immunostaining. The study was approved by the Alexandria University, Faculty of Medicine Research Ethics Committee. The age of the patients ranged between 27 and 90 years (Mean = 51, standard deviation = 10). Clinical data of all cases were obtained from the original pathology reports archived in the Pathology Department. The clinicopathological characteristics of the studied cases are summarized in Table 1.

Tissue Microarray (TMA) Construction

The formalin-fixed paraffin-embedded blocks of all cases were obtained from the archives of the Pathology Department for IHC studies. After examining the hematoxylin and eosin stained tumor tissue sections, microarray blocks were constructed to include two morphologically representative tumor areas (two tissue cylinders of 1 mm diameter punched out from the selected areas to be arrayed into the recipient block) selected for each tumor donor block. After confirming the adequacy of sampling in the microarray blocks, 4 μ thick serial sections were cut and mounted on Superfrost/Plus slides (Thermo Scientific, USA) for IHC staining.

IHC Staining

The TMA sections were incubated overnight with the primary antibodies purchased from Lab Vision, Fremont, California, USA; ER and PR, rabbit monoclonal antibodies clones (SP1), and (SP2), respectively, at 1:300, HER2 Ab-17, mouse monoclonal clone (e2-4001+3B5) at 1:300, and Ki-67 rabbit polyclonal (ready to use), after heat induced antigen retrieval according to the manufacturer's protocol and blocking endogenous peroxidase activity using 3% hydrogen peroxide. The universal kit used was anti-polyvalent, horseradish peroxidase/DAB, ready-to-use (Thermo Scientific, USA). Immunostainings were manually processed, with appropriate positive (breast carcinoma for ER, PR, HER2-neu, and Ki-67) and negative (omission of the primary antibody) controls included in each run.

Scoring of the Immunostained Slides

All cases were scored at ×40 magnification using an Olympus microscope. ER and PR scoring was done following the Allred score with >1% staining of tumor cell nuclei scored as positive [22]. Interpretation of HER2 was done following the

Table 1. Distribution of Ki-67 across the different clinicopathological factors among the 164 studied breast carcinomas

Clinicopathological factors	*N*	Ki-67 (*n* = 164)		Ki-67 ≥ 14 *(n = 135)*	Ki-67 ≥ 20 *(n = 128)*	*P* value
		MdSn (IQR)		*N (%)*	*N (%)*	
ER						
Negative	48	72	(46)	41 (85)	40 (85)	$(0.763)^{P_c}$
Mildly positive	20	60	(77)	15 (75)	14 (70)	$(0.788)^{P_{14}}$
Moderately positive	39	80	(45)	32 (82)	31 (79)	$(0.612)^{P_{21}}$
Severely positive	57	69	(73)	47 (82)	43 (75)	$(0.965)^{P_c}$
ER						
Negative	48	72	(46)	41 (85)	40 (83)	$(0.503)^{P_{14}}$
Positive	116	70	(70)	94 (81)	88 (76)	$(0.293)^{P_{21}}$
PR						
Negative	55	76	(40)	48 (87)	48 (87)	$(0.445)^{P_c}$
Mildly positive	25	53	(70)	18 (72)	16 (64)	$(0.375)^{P_{14}}$
Moderately positive	45	70	(64)	38 (84)	35 (78)	$(0.115)^{P_{21}}$
Severely positive	39	69	(75)	31 (79)	29 (74)	$(0.083)^{P_c}$
PR						
Negative	55	76	(40)	48 (87)	48 (87)	$(0.237)^{P_{14}}$
positive	109	70	(75)	87 (80)	80 (73)	$(0.047)^{P_{21}}$
HER 2						$(0.280)^{P_c}$
Negative	136	70	(67)	109 (80)	104 (76)	$(0.084)^{P_{14}}$
Positive	28	79	(45)	26 (93)	24 (86)	$(0.282)^{P_{21}}$
Molecular type						
Luminal A	25	05	(8)	0 (0)	0 (0)	$(<0.001)^{P_c}$
TN	24	78	(39)	22 (92)	22 (92)	$(<0.001)^{P_{14}}$
HER2 enriched	9	85	(55)	8 (89)	8 (89)	$(<0.001)^{P_{21}}$
Luminal B HER2-positive	19	78	(41)	18 (95)	16 (84)	$(0.811)^{P_c}$
Luminal B HER2-negative	87	77	(30)	87 (100)	82 (94)	$(0.684)^{P_{14}}$
Tumor size						
T1 or T2	102	70	(69)	83 (81)	78 (76)	$(0.531)^{P_{21}}$
T3 or T4	62	70	(49)	52 (84)	50 (81)	$(0.771)^{P_c}$
LN status						
No	32	72	(76)	24 (75)	24 (75)	$(0.227)^{P_2}$
Yes	132	70	(60)	111 (84)	104 (79)	$(0.642)^{P_{21}}$
Histological type						
IDC	150	70	(55)	125 (83)	119 (79)	$(0.094)^{P_c}$
ILC	5	55	(75)	3 (60)	3 (60)	$(0.388)^{P_{14}}$
Mixed	7	22	(60)	6 (86)	5 (71)	$(0.531)^{P_{21}}$
Histological grade						$(0.700)^{P_c}$
G1 or G2	129	70	(60)	107 (83)	102 (79)	$(0.685)^{P_{14}}$
G3	35	67	(75)	28 (80)	26 (74)	$(0.544)^{P_{21}}$
Mitotic index						
Score 1	60	61	(74)	45 (75)	43 (72)	$(0.160)^{P_c}$
Score 2	96	75	(40)	85 (89)	81 (84)	$(0.031)^{P_{14}}$
Score 3	8	38	(78)	5 (63)	4 (50)	$(0.025)^{P_{21}}$
LV invasion						$(0.631)^{P_c}$
Detected	97	70	(50)	82 (85)	80 (82)	$(0.370)^{P_{14}}$
Not-detected	67	70	(75)	53 (79)	48 (72)	$(0.099)^{P_{21}}$

* ^{P_c} stands for the *P* value testing if there is any statistically significant difference in the continuous Ki-67 distribution. $^{P_{14}}$ stands for the *P* value testing if there is any statistically significant difference in the Ki-67 distribution categorized at a cutoff value of 14%. $^{P_{12}}$ stands for the *P* value testing if there is any statistically significant difference 2win the Ki-67 distribution categorized at a cutoff value of 20%. ILC: Infiltrating lobular carcinoma, IDL: Infiltrating ductal carcinoma, LV: Lymphovascular, LN: Lymph node

guidelines of the American Society of Clinical Oncology/ College of American Pathologists with a positive HER2 result being IHC staining of 3+ (uniform, intense membrane staining of >10% of invasive tumor cells) or with an amplification ratio for fluorescent *in situ* hybridization of 2.0 or more being the cut point that was used to segregate immunohistochemistry equivocal tumors (scored

as 2+) [23]. The Ki-67 index was defined as the percentage of a total number of tumor cells with nuclear staining according to the recommendations of the International Ki-67 in BC Work Group [12], with a cutoff of ≥14% being regarded as Ki-67 overexpression to differentiate luminal A from luminal B HER2-negative tumors [24].

Thus, cases were categorized as luminal A subtype when: ER and/or PR positive, HER2 negative, and Ki-67 low (Ki-67 index of <14%), as luminal B-HER2 negative when: ER and/or PR positive, HER2 negative, and Ki-67 high (Ki-67 index of ≥14%), as luminal B-HER2 positive cases when: ER and/or PR positive, HER2 positive, with any Ki-67 index, as HER2 enriched when: ER and PR negative, HER2 positive, Ki-67 low or high, and as triple negative (TN) when: ER, PR, and HER2 negative, Ki-67 low or high.

Statistical Analysis

Quantitative data were described using median and interquartile range (IQR). Qualitative data were described using number and percentage. As the distribution of Ki-67 was deviated from normal, nonparametric Mann–Whitney *U* and Kruskal–Wallis tests were used to compare the distribution of Ki-67 between two and more than two groups, respectively. Correlations between two quantitative variables were tested using Spearman's correlation coefficient.

To test the effect of the interaction between the molecular subtypes and some clinicopathological factors on the Ki-67 labeling index, nonparametric rank-based estimation for linear models was used. In the case of a statistically significant interaction, *post-hoc* comparisons with Bonferroni correction were conducted unless the sample size was too small.

"Rfit," a statistical R package [25], was used to test the interaction and SPSS® Statistics 20 was used to conduct the other statistical tests.

Results

The clinicopathological characteristics of the 164 studied breast carcinomas are shown in Table 1. The median age of patients was 50 years (range: 20-91 years). The majority of the tumors were pT2 (59%). In total, 32/164 cases (19.5%) were LN negative. Grade II tumors accounted for 68.9% (113/164) of cases. The ER, PR, and HER2 positive rates were 70.7% (116/164), 66.5% (109/164), and 17% (28/164), respectively. The median Ki-67 score was 25% (IQR: 64%; range: 0-91%) [Figure 1].

Of the 164 tumors, 25 (15.3%) were classified as luminal A subtype, 87 (53.1%) as luminal B HER2negative, 19 (11.6%) as luminal B HER2positive, 9 (5.4%) as HER2enriched, and 24 (14.6%) as TN.

Association between Ki-67 and Clinicopathological Parameters

As shown in Table 1, as a continuous variable, the median Ki-67 score did not show significant differences with the HR status, HER2 positivity, tumor grade, tumor size, LN status, and lymphovascular invasion. It was lower in infiltrating lobular than in infiltrating ductal carcinoma cases and was the least in cases showing mixed histological type.

When Ki-67 was categorized into high and low expression groups, at a cutoff ≥ 14%, it correlated significantly as a categorized variable only with the mitotic index ($P = 0.025$), but when a cutoff ≥ 20 was used, an additional significant association was noted between Ki-67 index and the PR status ($P = 0.047$).

As shown in Table 1, the median Ki-67 score was the highest (85%) in the HER2 enriched subtype, followed by the luminal B HER2-positive and TN subtypes (78% for both), then the luminal B HER2-negative (77%), and was the lowest among luminal A tumors (5%). Pairwise comparisons showed a statistically significant difference between luminal A and all other subtypes ($P < 0.001$); the differences between all other pairs were not statistically significant [Figure 2].

Ki-67 Expression within the Molecular Subtypes with Different Tumor Grades, Tumor Sizes, and LN Statuses

The changes in the distribution of Ki-67 expression within the different molecular subtypes with the

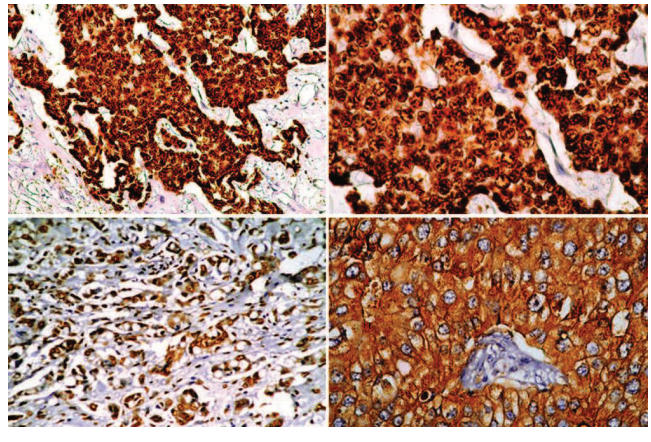

Figure 1. Strong diffuse nuclear staining for (a) estrogen receptor (×400), (b) progesterone receptor (×400), (c) Ki-67 (high index), (×400); and in (d) strong HER2 complete membranous staining in >10% of tumor cells (×400)

various tumor grades, tumor sizes, and LN statuses are demonstrated in Table 2.

The median Ki-67 level in T1 or T2 tumors compared to that in T3 or T4 tumors were slightly higher in luminal B HER2-positive cases, slightly lower in HER2 enriched cases, and nearly similar among luminal A, TN and luminal B HER2-negative subtypes. Despite this, the differences in the Ki-67 distribution between T1 or T2 and T3 or T4 cases among the different molecular types did not reach statistical significance ($P = 0.674$) [Figure 3].

Table 2 and Figure 4 demonstrate the Ki-67 distribution changes between N0 or N1 and N2 or N3 cases. This change varied among the different molecular types ($P < 0.001$). Post-hoc comparisons were conducted to test the change of Ki-67 distribution between N0 or N1 and N2 or N3 cases among three molecular subtypes only: Luminal A, luminal B HER2-negative, and TN. As there were only two cases observed among the HER2 enriched N0 or N1 and luminal B HER2-positive N0 or N1 groups, post-hoc pairwise comparisons were not conducted in these two groups. The median Ki-67 was higher, but not statistically significant, in N0 or N1 group compared to that in N2 or N3 in luminal A ($U = 51$; $P = 0.696$), luminal B HER2-positive and luminal B HER2-negative cases ($U = 408.5$; $P = 0.139$). This observation was reversed among the HER2- enriched and TN cases ($U = 39.5$, $P = 0.202$). The P values of the post-hoc comparisons were compared to an adjusted α level of 0.0167 instead of 0.05.

Table 2 and Figure 5 also demonstrate that the median Ki-67 was lower in G1 or G2 tumors compared to that in G3 in all molecular types except in luminal B HER2-positive tumors where the median Ki-67 was lower in G3 cases. Yet, this observation was not statistically significant ($P = 0.687$).

Discussion

Assessment of tumor cell proliferation is mandatory in the pathological evaluation of all breast tumors, and is accomplished simply through the assessment of the mitotic activity, a pivotal component of tumor grading. The role of Ki-67 IHC as a biomarker in BC is still being investigated, as consensus has not been reached to justify its inclusion in routine clinical practice [26].

Despite that, the baseline elevated Ki-67 has been shown in an Italian study to be associated with complete pathological and clinical response [27], yet others reported a statistically significant correlation between a high Ki-67 and an increased risk of cancer relapse and death in BC patients [18]. Furthermore, the Ki-67 level at 2 weeks of treatment was concluded to be a better predictor of the response to endocrine therapy and recurrence-free survival in ER positive BC patients than the pretreatment levels [28-30]. Conversely, Learn *et al*. [31] did not demonstrate any statistically significant association of Ki-67 index with the clinical response rate. Thus, the evidence supporting the

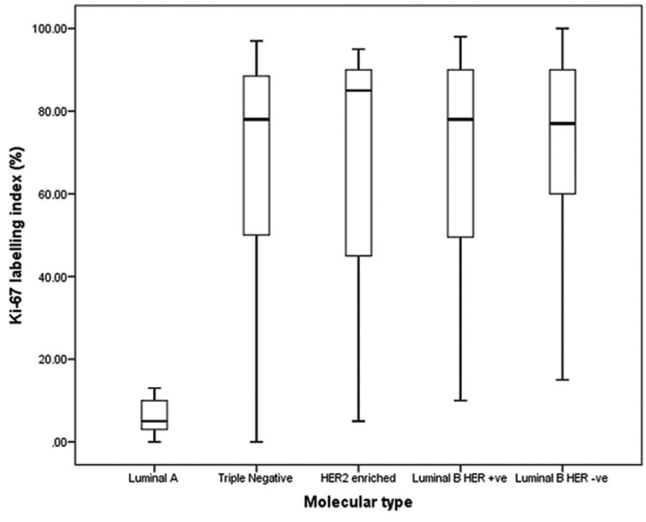

Figure 2. Distribution of the Ki-67 labeling index among the different molecular subtypes of the studied breast carcinoma cases

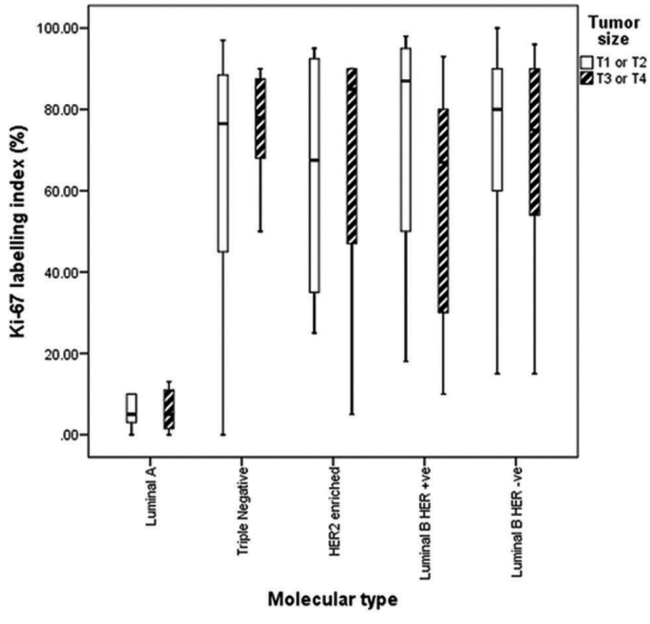

Figure 3. Change in the distribution of Ki-67 among the different molecular subtypes and tumor size

clinical utility of Ki-67 is insufficient to justify its implementation as a routine prognostic biomarker in newly diagnosed BCs [32].

It is also plausible that the significance of Ki-67 in isolation cannot be confirmed because of the absence of standardized guidelines for the fixation of the specimens, differences in the antibody clones used and in the counting procedures as well as differences in the cutoffs used to stratify high or low Ki-67 expression. Therefore, the clinical utility of Ki-67 might be enhanced if it is assessed in association with other prognostic factors in more narrowly defined tumor subgroups.

In this study, the median Ki-67 value was 25% which is similar to the median of 20.0-22.7% reported by others [33,34] yet higher than the 12% that was reported by Viale *et al*. [35]. The median Ki-67 expression level was the highest among the HER2 enriched subtype, the lowest among luminal A tumors, and did not differ much among the luminal B and TN subtypes. Statistically significant differences were noted regarding the Ki-67 index among the different molecular subtypes. These results indicate that combined HR negativity and HER2 positivity confer an increased proliferative activity to BC cells, and thereby Ki-67 represents an accurate biomarker that reflects tumor cell proliferative activity. These findings differ slightly from the previous reports that demonstrated higher Ki-67 expression levels in the TN and HER2-positive subtypes compared with the luminal subtypes [36], as among our cases Ki-67 did not reveal this difference between the TN and luminal B tumors.

The previous studies that investigated the correlation between

Ki-67 and clinicopathological parameters reported controversial findings [26,37]. One study revealed a significant association between Ki-67 and tumor grade, PR, HER2, and LN status [26]. Other studies [11,33] demonstrated an association

Table 2. Change in the distribution of Ki-67 among the different molecular subtypes according to tumor size, LN status, and histological tumor grade

Group	Tumor size		Nodal involvement		Grade	
	T1 or T2	T3 or T4	N0 or N1	N2 or N3	G1 or G2	G3
Luminal A						
N	17	8	6	19	18	7
Ki-67 Median (IQR)	0.05 (0.07)	0.05 (0.10)	0.08 (0.06)	0.05 (0.08)	0.05 (0.07)	0.1 (0.11)
Ki-67 ≥ 14 (*N*)	0	0	0	0	0	0
Ki-67 ≥ 20 (*N*)	0	0	0	0	0	0
TN						
N	16	8	7	17	17	7
Ki-67 Median (IQR)	0.77 (0.45)	0.78 (0.24)	0.70 (0.55)	0.80 (0.35)	0.75 (0.40)	0.80 (0.29)
Ki-67 ≥ 14 (*N*)	14	8	6	16	15	7
Ki-67 ≥ 20 (*N*)	14	8	6	16	15	7
HER2 enriched						
N	4	5	2	7	6	3
Ki-67 Median (IQR)	0.68 (0.64)	0.85 (0.64)	0.15[a]	0.90 (0.43)	0.68 (0.71)	0.85[a]
Ki-67 ≥ 14 (*N*)	4	4	1	7	5	3
Ki-67 ≥ 20 (*N*)	4	4	1	7	5	3
Luminal B HER2+						
N	10	9	2	17	15	4
Ki-67 Median (IQR)	0.87 (0.45)	0.67 (0.63)	0.82[a]	0.78 (0.52)	0.80 (0.41)	0.59 (0.63)
Ki-67 ≥ 14 (*N*)	10	8	2	16	14	4
Ki-67 ≥ 20 (*N*)	9	7	2	14	13	3
Luminal B HER2-						
N	55	32	15	72	73	14
Ki-67 Median (IQR)	0.80 (0.30)	0.75 (0.37)	0.87 (0.22)	0.73 (0.34)	0.75 (0.30)	0.84 (0.39)
Ki-67 ≥ 14 (*N*)	55	32	15	72	73	14
Ki-67 ≥ 20 (*N*)	51	31	15	67	69	13
Statistical test						
P_1	(< 0.001)		(< 0.001)		(< 0.001)	
P_2	(0.582)		(0.003)		(0.842)	
P_3	(0.674)		(< 0.001)		(0.687)	

P_1 stands for the P value testing whether Ki-67 distribution differed with the molecular subtype. P_2 stands for the *P* value testing whether Ki-67 distribution differed with different tumor sizes, LN statuses or histologic tumor grades. P_3 stands for the *P* value testing whether the change in the ki-67 distribution among different tumor sizes, LN statuses or tumor grades differed with the molecular subtype. [a]The range instead of the interquartile range was presented as the number of cases was small. LN: Lymph node, IQR: Interquartile range

between higher Ki-67 index (at cutoff >20% and >19%, respectively) and higher tumor grade, larger tumor size, positive nodes, and HR. Conversely, in this study, the Ki-67 expression levels did not exhibit any significant association with tumor histological type, grade, tumor size, and nodal status. These differences may be attributed to differences in the studied population, as their population presented with less advanced tumors. Furthermore, the use of TMA which might not allow proper Ki-67 assessment - as whole sections - if Ki-67 is heterogeneously expressed could justify these differences. However, our results are in agreement with another Egyptian study [38], which reflects the importance of Ki-67 as an indicator of tumor biology and aggressiveness rather than of tumor stage, which is expected to be upgraded with a lack of awareness and underdevelopment of the health-care systems.

In an attempt to discover whether the change in the distribution of Ki-67 with tumor size, LN status, and histological tumor grade differs among the different molecular subtypes or not, we studied the interaction effect between the molecular subtypes and some clinicopathological factors (tumor size, LN status, and tumor grade) on the distribution of Ki-67.

Our results demonstrated that the Ki-67 score was slightly higher among T1/T2 compared to T3/T4 luminal B HER2-positive tumors, and slightly lower in HER2 enriched cases. Although both molecular types feature HER2 positivity, yet the tumor proliferative activity exhibited different patterns when examined in relation to the T-stage. It can be suggested that the luminal B HER2- positive tumors progress to a certain stage beyond which the Ki-67 score does not increase accordingly. This may be related to inadequate tumor vascularity, which is unable to support tumor growth after a certain point.

The fact that this did not apply for HER2 enriched tumors, which showed slightly higher proliferation in T3/T4 compared to T1/T2 tumors, may be explained by the increased expression of HER2-associated genes [39] that act unopposed by the ER and PR negativity. Our finding that among luminal A, luminal B HER2-negative, and TN cases the proliferation was nearly similar among T1/T2 and T3/T4 tumors may be attributed to the greater number of T1/T2 compared to T3/T4 tumors among those subtypes.

Conversely, one previous study [40] revealed that the mean Ki-67 scores were not significantly different between the HER2-positive (non-luminal) and TN subtypes, with the exception of patients with a tumor size of >2 cm, which indicated the presence of a stronger proliferative activity in the TN subtype compared with the HER2positive (non-luminal) subtype, with regard to patients with a tumor size of >2 cm BC patients.

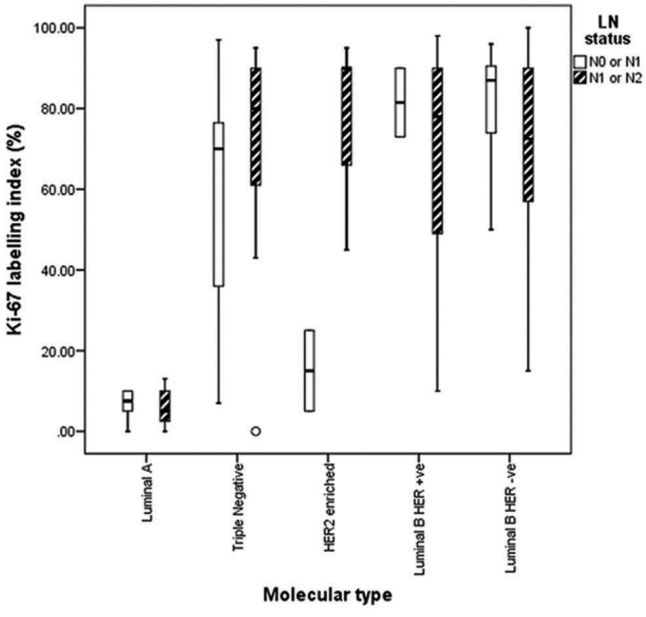

Figure 4. Change in the distribution of Ki-67 among the different molecular subtypes and the LN status. Note that only two cases were observed among HER2 enriched N0 or N1 and luminal B HER2+ve N0 or N1 groups

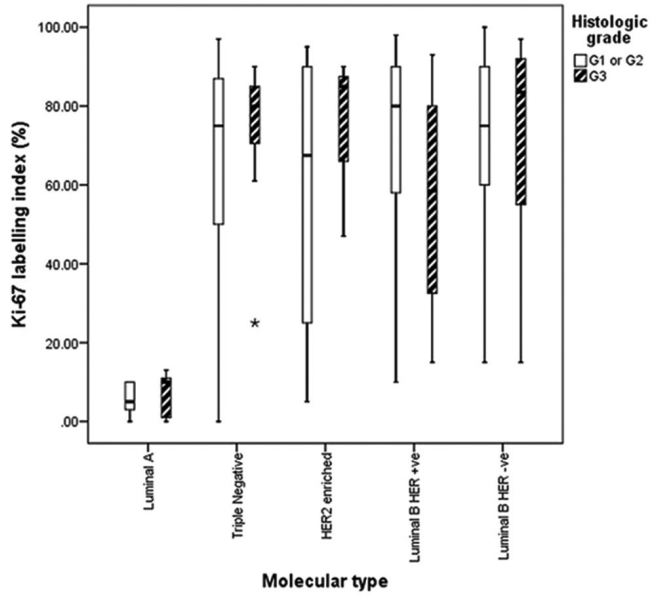

Figure 5. Change in the distribution of Ki-67 among the different molecular subtypes and the histologic grade of the tumor. Note that the number of cases in Grade-3 HER2-enriched tumors was only 3

Although the results of this study revealed the absence of significant differences between the Ki-67 scores and the ER, PR, and HER2-status, yet it was noted that among our cases the differences of Ki-67 distribution between N0 or N1 and N2 or N3 varied significantly among the different molecular types ($P < 0.001$). The median Ki-67 was higher in N0 or N1 group compared to that in N2 or N3 among luminal A, luminal B HER2-positive and luminal B HER2-negative cases. This observation was reversed among HER2-enriched and TN cases. Due to the limitation of the sample size, pairwise comparisons were not conducted in the HER2 enriched and luminal B HER2- positive subtypes. The other three post-hoc comparisons were not statistically significant, a finding that can be attributed to the small sample size. These results signify that the proliferative activity of BC cells is high with the lower levels of ER and PR, or higher levels of HER2.

Thus, our results suggest that among luminal tumors, the proliferative activity increases until nodal metastasis develops, and then it does not increase proportionately with the increase in the nodal stage, which reflects the importance of assessing tumor proliferation in this subset of tumors as the metastatic potential of these luminal tumors may be partly dependent on the proliferative activity. Conversely, the metastatic potential of non-luminal tumors might be attributed to factors other than the proliferative activity, probably the HR negativity.

In conclusion, though the prognostic and predictive value of Ki-67 index in breast carcinoma differ among studies, yet, our results highlight that the clinical utility of Ki-67 increases when interpreted within the context of the molecular subtypes in combination with other prognostic markers. Our findings also suggest an impact of Ki-67 as a proliferation marker on the biological behavior of tumors that is context dependent. We think that the level of statistical significance could have been reached if the sample size was larger within the molecular subtypes other that the luminal B HER2-negative. Thus, further research with larger sample size is recommended to shed the light on how the molecular types can affect the relation between Ki-67 and the different clinicopathological characteristics, particularly the LN status. We strongly recommend the IHC4 panel (ER, PR, HER2, and Ki-67) to be used in the initial assessment of all newly diagnosed breast carcinomas, as the cost of adding Ki-67 to the routine triplereceptor panel is small but worthy, as the management of breast carcinomas should be molecular subtype-oriented.

Acknowledgments

The authors acknowledge all the technicians at the Pathology Labs, Alexandria Faculty of Medicine, and all the working staff at the archives of the Department of Pathology, Alexandria Faculty of Medicine, Alexandria, Egypt.

References

[1] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN Cancer Incidence and Mortality Worldwide. IARC Cancer Base No. 10. Vol. 1.2. Lyon, France: International Agency for Research on Cancer; 2008. Available from: http://www.globocan. iarc.fr/factsheets.2010. [Last accessed on 2017 Jan 20].

[2] Ozmen V. Breast cancer in the world and Turkey. J Breast Health 2008;4:7-12.

[3] Lin CH, Liau JY, Lu YS, Huang CS, Lee WC, Kuo KT, *et al*. Molecular subtypes of breast cancer emerging in young women in Taiwan: Evidence for more than just westernization as a reason for the disease in Asia. Cancer Epidemiol Biomarkers Prev 2009;18:1807-14.

[4] Alco G, Bozdogan A, Selamoglu D, Pilanci KN, Tuzlali S, Ordu C, *et al*. Clinical and histopathological factors associated with Ki-67 expression in breast cancer patients. Oncol Lett 2015;9:1046-54.

[5] Berry DA, Cronin KA, Plevritis SK, Fryback DG, Clarke L, Zelen M, *et al*. Effect of screening and adjuvant therapy on mortality from breast cancer. N Engl J Med 2005;353:1784-92.

[6] Berry DA, Cirrincione C, Henderson IC, Citron ML, Budman DR, Goldstein LJ, *et al*. Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. JAMA 2006;295:1658-67.

[7] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.

[8] Milde-Langosch K, Karn T, Müller V, Witzel I, Rody A, Schmidt M, *et al*. Validity of the proliferation markers Ki67, TOP2A, and RacGAP1 in molecular subgroups of breast cancer. Breast Cancer Res Treat 2013;137:57-67.

[9] Wirapati P, Sotiriou C, Kunkel S, Farmer P, Pradervand S, Haibe-Kains B, *et al*. Meta-analysis of gene expression profiles in breast cancer: Toward a unified understanding of breast cancer subtyping and prognosis signatures. Breast Cancer Res 2008;10:R65.

[10] Clahsen PC, van de Velde CJ, Duval C, Pallud C, Mandard AM, Delobelle-Deroide A, *et al*. The utility of mitotic index, oestrogen receptor and Ki-67 measurements in the creation of novel prognostic indices for node-negative breast cancer. Eur J Surg Oncol 1999;25:356-63.

[11] Viale G, Giobbie-Hurder A, Regan MM, Coates AS, Mastropasqua MG, Dell'Orto P, *et al*. Prognostic and predictive value of centrally reviewed Ki-67 labeling index in postmenopausal women with endocrineresponsive breast cancer: Results from Breast International Group Trial 1-98 comparing adjuvant tamoxifen with letrozole. J Clin Oncol 2008;26:5569-75.
[12] Dowsett M, Nielsen TO, A'Hern R, Bartlett J, Coombes RC, Cuzick J, *et al*. Assessment of Ki67 in breast cancer: Recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 2011;103:1656-64.
[13] Tovey SM, Witton CJ, Bartlett JM, Stanton PD, Reeves JR, Timothy G, *et al*. Outcome and human epidermal growth factor receptor (HER) 1-4 status in invasive breast carcinomas with proliferation indices evaluated by bromodoxyuridine labeling. Breast Cancer Res 2004;6:246-51.
[14] Tashima R, Nishimura R, Osako T, Nishiyama Y, Okumura Y, Nakano M, *et al*. Evaluation of an optimal cut-off point for the Ki-67 index as a prognostic factor in primary breast cancer: A retrospective study. PLoS One 2015;10:e0119565.
[15] Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ; Panel members. Strategies for subtypes - dealing with the diversity of breast cancer: Highlights of the St. Gallen International Expert Consensus on the primary therapy of early breast cancer 2011. Ann Oncol 2011;22:1736-47.
[16] Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, *et al*. Personalizing the treatment of women with early breast cancer: Highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2013. Ann Oncol 2013;24:2206-23.
[17] Stuart-Harris R, Caldas C, Pinder SE, Pharoah P. Proliferation markers and survival in early breast cancer: A systematic review and meta-analysis of 85 studies in 32,825 patients. Breast 2008;17:323-34.
[18] de Azambuja E, Cardoso F, de Castro G Jr, Colozza M, Mano MS, Durbecq V, *et al*. Ki-67 as prognostic marker in early breast cancer: A meta-analysis of published studies involving 12,155 patients. Br J Cancer 2007;96:1504-13.
[19] Tamaki K, Moriya T, Sato Y, Ishida T, Maruo Y, Yoshinaga K, *et al*. Vasohibin-1 in human breast carcinoma: A potential negative feedback regulator of angiogenesis. Cancer Sci 2009;100:88-94.
[20] Trihia H, Murray S, Price K, Gelber RD, Golouh R, Goldhirsch A, *et al*. Ki-67 expression in breast carcinoma: Its association with grading systems, clinical parameters, and other prognostic factors - A surrogate marker? Cancer 2003;97:1321-31.
[21] Goldhirsch A, Ingle JN, Gelber RD, Coates AS, Thürlimann B, Senn HJ; Panel members. Thresholds for therapies: Highlights of the St Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. Ann Oncol 2009;20:1319-29.
[22] Hammond ME, Hayes DF, Wolff AC, Mangu PB, Temin S. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. J Clin Oncol 2010;28:2784-95.
[23] Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, *et al*. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013;31:3997-4013.
[24] Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, *et al*. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 2009;101:736-50.
[25] Kloke JD, McKean JW. Rfit: Rank-based estimation for linear models. R J 2012;4:57-64, 369. Available from: https://www.journal.r-project. org/archive/2012-2/RJournal_2012-2_Kloke+McKean.pdf. [Last accessed on 2017 Jan 20].
[26] Haroon S, Hashmi AA, Khurshid A, Kanpurwala MA, Mujtuba S, Malik B, *et al*. Ki67 index in breast cancer: Correlation with other prognostic markers and potential in Pakistani patients. Asian Pac J Cancer Prev 2013;14:4353-8.
[27] Bottini A, Berruti A, Brizzi MP, Bersiga A, Generali D, Allevi G, *et al*. Cytotoxic and antiproliferative activity of the single agent epirubicin versus epirubicin plus tamoxifen as primary chemotherapy in human breast cancer: A single-institution phase III trial. Endocr Relat Cancer 2005;12:383-92.
[28] Smith IE, Dowsett M, Ebbs SR, Dixon JM, Skene A, Blohmer JU, *et al*. Neoadjuvant treatment of postmenopausal breast cancer with anastrozole, tamoxifen, or both in combination: The Immediate Preoperative Anastrozole, Tamoxifen, or Combined with Tamoxifen (IMPACT) multicenter double-blind randomized trial. J Clin Oncol 2005;23:5108-16.
[29] Dowsett M, Smith IE, Ebbs SR, Dixon JM, Skene A, Griffith C, *et al*. Proliferation and apoptosis as markers of benefit in neoadjuvant endocrine therapy of breast cancer. Clin Cancer Res 2006;12:1024s-30.
[30] Dowsett M, Procter M, McCaskill-Stevens W, de Azambuja E, Dafni U, Rueschoff J, *et al*. Disease-free survival according to degree of HER2 amplification for patients treated with adjuvant chemotherapy with or without 1 year of trastuzumab: The HERA Trial. J Clin Oncol 2009;27:2962-9.

[31] Learn PA, Yeh IT, McNutt M, Chisholm GB, Pollock BH, Rousseau DL Jr, *et al*. HER-2/neu expression as a predictor of response to neoadjuvant docetaxel in patients with operable breast carcinoma. Cancer 2005;103:2252-60.

[32] Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, *et al*. American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 2007;25:5287-312.

[33] Nishimura R, Osako T, Okumura Y, Hayashi M, Toyozumi Y, Arima N. Ki-67 as a prognostic marker according to breast cancer subtype and a predictor of recurrence time in primary breast cancer. Exp Ther Med 2010;1:747-54.

[34] Nishimura R, Osako T, Okumura Y, Tashima R, Toyozumi Y, Arima N. Changes in the ER, PgR, HER2, p53 and Ki-67 biological markers between primary and recurrent breast cancer: Discordance rates and prognosis. World J Surg Oncol 2011;9:131.

[35] Viale G, Regan MM, Dell'Orto P, Mastropasqua MG, Maiorano E, Rasmussen BB, *et al*. Which patients benefit most from adjuvant aromatase inhibitors? Results using a composite measure of prognostic risk in the BIG 1-98 randomized trial. Ann Oncol 2011;22:2201-7.

[36] Mitrovic O, Cokic V, Đikic D, Budec M, Vignjevic S, Suboticki T, *et al*. Correlation between ER, PR, HER-2, Bcl-2, p53, proliferative and apoptotic indexes with HER-2 gene amplification and TOP2A gene amplification and deletion in four molecular subtypes of breast cancer. Target Oncol 2014;9:367-79.

[37] Pinder SE, Wencyk P, Sibbering DM, Bell JA, Elston CW, Nicholson R, *et al*. Assessment of the new proliferation marker MIB1 in breast carcinoma using image analysis: Associations with other prognostic factors and survival. Br J Cancer 1995;71:146-9.

[38] Elzawahry HM, Saber MM, Mokhtar NM, Zeeneldin AA, Ismail YM, Alieldin NH. Role of Ki67 in predicting resistance to adjuvant tamoxifen in postmenopausal breast cancer patients. J Egypt Natl Canc Inst 2013;25:181-91.

[39] Marcom PK, Isaacs C, Harris L, Wong ZW, Kommarreddy A, Novielli N, *et al*. The combination of letrozole and trastuzumab as first or secondline biological therapy produces durable responses in a subset of HER2 positive and ER positive advanced breast cancers. Breast Cancer Res Treat 2007;102:43-9.

[40] Sun J, Chen C, Wei W, Zheng H, Yuan J, Tu YI, *et al*. Associations and indications of Ki67 expression with clinicopathological parameters and molecular subtypes in invasive breast cancer: A population based study. Oncol Lett 2015;10:1741-8.