Ki67 expression in breast cancer

Correlation with prognostic markers and clinicopathological parameters in Saudi patients

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ABSTRACT

الأهداف: تقييم نمط الظهور المناعي لصبغة Ki67 في مرضى سرطان الثدي السعوديات وفحص أي قيمة تنبؤية أو تكهنية ممكنة ل Ki67.

الطريقة: هذه دراسة استعادية صممت بهدف تقييم كمي لمؤشر Ki67 (Ki67 PI) التكاثري في عينات أورام الثدي من 115 مريضة سعودية شُخصت بين يناير ومارس 2015 بقسم الباثولوجي، مستشفى الملك فهد، المدينة المنورة، المملكة العربية السعودية. وربطت العلاقة بين نتائج (Ki67 PI) مع النتائج الفردية والمشتركة للصبغات المناعية، PR، ER وكذلك العوامل الاكلينيكية المرضية في (HER2)/neu هذه الحالات.

النتائج: ظهر النشاط المناعي Ki67 بوضوح عالي في 85 مريضة بنسبة 73.9%، وإرتبط (Ki67 PI) مع العوامل الإكلينيكية المرضية السيئة التنبؤ كتقدم سن المريضة وارتفاع درجة الورم وظهور أورام ثانوية في العقد الليمفاوية وكذلك زيادة إيجابية صبغة Her-2/neu بقيم (p<0.02, p<0.01, p<0.001, p<0.009) على التوالي. وقد تم الكشف عن إرتفاع (Ki67 PI) في 62.9% من الأورام الفرعية A و76.1% من الأورام االفرعية القاعدية و84.4% من الأورام الفرعية B و100% من الأورام الفرعية Her-2. كما ارتبط بشكل مؤثر مع الأنواع الفرعية الجزيئية لسرطان الثدي التي اشتملت على إيجابية Her-2/neu مثل الأورام الفرعية B والأورام الفرعية Her-2 مقارنة بالأورام الفرعية A والتى بها Her-2/neu سالب الظهور بقيمة (p<0.04).

الخاتمة: وُجد أن المؤشر التكاثري Ki67 لدى مريضات سرطان الثدي السعوديات أعلى بكثير مقارنة بما سجل فيما تم نشره عالمياً. كما ظهر أن أعلى نسبة له كانت في أنواع سرطان الثدي الجزيئية الفرعية Her-2 والفرعية B. ووُجد أنه يمكن استخدام المؤشر التكاثري Ki67 بالمشاركة مع مؤشرات التكهن الأخرى كوسيلة في إدارة علاج مريضات سرطان الثدي السعوديات. وينبغي استخدام (Ki67 PI) بصورة روتينية في فحص أورام الثدي في مختبرات الأنسجة والأورام.

Objectives: To evaluate Ki67 immunoexpression pattern in Saudi breast cancer (BC) patients and investigate any possible predictive or prognostic value for Ki67.

Methods: This is a retrospective study designed to quantitatively assess the Ki67 proliferative index (PI) in retrieved paraffin blocks of 115 Saudi BC patients diagnosed between January 2005 and March 2015 at the Department of Pathology, King Fahd Hospital, Al Madinah Al Munawarah, Kingdom of Saudi Arabia. The Ki67 PI was correlated with individual and combined immunoprofile data of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu) with their clinicopathological parameters.

Results: Ki67 immunoreactivity was highly expressed (>25% of the tumor cells were positive) in 85 (73.9%) patients. The Ki67 PI was significantly associated with poor prognostic clinicopathological parameters including old age (p<0.02), high tumor grade (p<0.01), lymph node metastasis (p<0.001), and Her-2/neu positivity (p<0.009). However, the association with ER positivity, PR positivity, tumor size, and lymphovascular invasion were not statistically significant. The Ki67 PI was significantly associated with BC molecular subtypes that were Her2/neu positive (luminal B and HER-2) subtypes compared with the Her2/neu negative (luminal A) subtype (p<0.04).

Conclusion: The Ki67 PI is significantly higher in Saudi BC patients comparing with the reported literature. Ki67 PI was highest in the HER-2 and luminal-B molecular subtypes. Along with other prognostic indicators, Ki67 PI may be useful in predicting prognosis and management of Saudi BC patients.

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Breast cancer (BC) is one of the most common malignancy in the world.[1] Although in the Kingdom of Saudi Arabia (KSA), the incidence of BC is much lower than in the Western world; it is still the most common malignancy in the Saudi women. According to the Saudi Cancer registry,[2] BC accounted for approximately 23% of all the newly diagnosed female cancers. An additional significant fact on BC in KSA is its special presentation; as it predominantly affects the younger population, frequently presents as higher histological grades and in advanced clinical stages.[2-4] Apart from the problem of its being highly prevalent globally and locally; BC has also shown its divergent nature with regards to its clinical course, response to treatment, and prognostic outcomes. Thus, the new molecular classification of BC has emerged on the basis of biomarkers. In the initial stages, the hormones namely, the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2) played their roles. It was only 15 years ago, that the molecular classification of BC was proposed by Californian scientists, initially there were 4 major classes: luminal-like, basal-like, normal-like, and Her2 positive.[5] Consequently, a fifth class was added, dividing one of the major luminal class to luminal A and luminal B classes.[6-8] Ki67 had been known to be an important proliferation biomarker since 1980s. It has recently become an essential component of routine biomarker profile for BC, along with ER, PR ,and Her2, to assist the oncologists in delivering optimum treatment to BC patients. Its role as a poor prognostic biomarker is well established, and a number of studies have found a significant correlation between Ki67 positivity with that of histological parameters such as nuclear grades and mitotic figures.[9] Recent studies have also proved its predictive role in both the antihormonal therapy and chemotherapy for the efficacy of the treatment. The aim of this study is to examine the Ki67 biomarker in the BC patients and the immunohistochemically on the paraffin embedded blocks. Subsequently, to correlate the Ki67 findings with individual and combined immunoprofile data of ER, PR, and Her2/neu, as well as with their clinicopathological parameters to identify any specific differences in our BC cases as compared with western cases. This is may be important in investigating any predictive or prognostic role of Ki67 in managing BC patients in KSA population.

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Methods.

Patient's tissue and data collection. A total of 115 patients with invasive BC diagnosed between January 2005 and March 2015 at the Department of Pathology, Al Madinah Al Munawarah, KSA were included in this study. Each case was reviewed by 2 authors and histologically graded with regard to a number of features (**Table 1**). Clinicopathological parameters, including age at diagnosis, tumor type, size, lymph node, and lymphovascular status were all available. Histologic grade was assessed by Modified Bloom-Richardson System (MBR). Only histopathologically confirmed invasive carcinoma cases were included. In-situ lesions, recurrences, biopsies, sarcomas, benign lesions, and metastases were excluded. Modified radical mastectomies, quadrectomy, and wide local excision specimens were included.

This study was approved for publication by the Pathology Department, King Fahad Hospital, Al Madinah Al Munawarah, KSA.

Molecular subtypes of breast cancer. The BC were classified into 4 subtypes as follows: luminal A (ER+, PR+/-, Her2/neu -), luminal B (ER+, PR+/-, Her2/neu +), Her2 (ER-, PR-, Her2/neu +), and basal-like (ER-, PR-, Her2-/neu-) according to Onitilo et al[10] (**Table 1**).

Ki67 immunohistochemical (IHC) staining. Tissue cores were extracted from archival blocks of the primary BC and used to construct a tissue miniarray (TmA) as previously described.[11] To overcome the problem of BC heterogeneity during measurement of Ki67 proliferative index (PI), 2 representative cancerous foci were marked on slides with hematoxylin and eosin-stained sections from the selected paraffin blocks. Two tissue cores (each of 2 mm) were used to create TmAs from each block. In most TmA modules, it is recommended to obtain at least 2 cores per tumor to improve its reliability.[12] Immunohistochemical staining of Ki-67 was performed at room temperature and washes were performed with tris-buffered saline. A 4 μm slices of formalin-fixed paraffin-embedded TmA were dewaxed and rehydrated. Antigen retrieval was carried out using EDTA retrieval solution PH 9 in a standard microwave oven for 10 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide (S2023, Dako, Glostrup, Denmark). Sections were then incubated with primary antibodies against Ki67 (MIB-1, 1:100, Dako, Glostrup, Denmark) for 32 minutes at 42°C according to the manufacturer's instructions. The signals were visualized with diaminobenzidine (K3468, Dako, Glostrup, Denmark) for 10 minutes, and were counterstained with hematoxylin.

Scoring of Ki67 immunostaining. The nuclear immunostaining of Ki67 was assessed by counting at

least 500 tumor cells per case across 5 high power fields of the section under microscope. The Ki67 PI was scored as high when ≥25% of the tumor cells were positive; and low when <25% cells were positive (**Figure 1**). The immunostained slides were evaluated independently.

Statistical analyses. Data were statistically analyzed using the Statistical Package for Social Sciences Version 22.0 for Windows (IBMCorp, Armonk, NY, USA). Interobserver reproducibility was assessed by correlation analysis between the 2 data sets. Association between the Ki67 PI and patient clinicopathological parameters was determined using the Chi-squared test and Fisher's exact test. For all statistical analyses, a *p*-value of ≤0.05 was considered significant.

Results. The clinicopathological characteristics of 115 female BC patients are shown in **Table 1**. The mean age was 50 years (range: 26-88 years). The pathological examination showed invasive ductal carcinoma, not otherwise specified in 109 (94.9%) cases, infiltrating lobular carcinoma, mucinous carcinoma, and metaplastic carcinoma in 2 (1.7%) cases each. Tumor grades distribution was grade I, grade II, and grade III. Tumor size distribution was T1 (15.6%); T2 (74%), and T3 (10.4%). Seventy-seven cases (67%) had positive lymph nodes and 83 cases (72.2%) had lymphovascular invasion (LVI).

Molecular classification of breast cancers. The percentage of PR positive BC was 62.7%, followed by ER positive (61.8%), and Her2/neu over-expression (38.2%). The most frequent BC subtype in the studied patients was luminal A (47%), followed by luminal B (27.8%), and basal-like subtypes (18.3%), whereas the percentage of Her2/neu subtype was 6.9% (**Table 1**). These data was collected from a study carried out by authors.[13]

Assessment of Ki67 immunostaining. The Ki67 staining was highly expressed (≥25%) in 85 (73.9%) BC patients (**Figure 1**). Ki67 expression was significantly associated with old age ($p<0.02$), high tumor grade ($p<0.01$), lymph node metastasis ($p<0.001$), and Her2/neu positivity ($p<0.009$) (**Table 2**). However, the association with ER positivity, PR positivity, tumor size, and LVI were not statistically significant. (**Table 2**). High expression of Ki67 protein was detected in 62.9% of luminal A tumors, 76.1% of basal-like tumors, 84.4% of luminal B tumors, and 100% of Her2/neu tumors. The average Ki67 PI was significantly different between luminal A, luminal B, and Her2/neu subtypes ($p<0.04$).

Table 1 - Clinicopathological and immunoprofile characteristics of 115 breast cancer cases.

Characteristics	Number (%)
Age (years)	
≤50	58 (50.4)
>50	57 (49.6)
Tumor type	
Invasive ductal carcinoma	109 (94.9)
Invasive lobular carcinoma	2 (1.7)
Mucinous carcinoma	2 (1.7)
Metaplastic carcinoma	2 (1.7)
Tumor grade	
Grade I	6 (5.2)
Grade II	58 (50.4)
Grade III	51 (44.4)
Tumor size	
T1: <2 cm	18 (15.6)
T2: 2-5 cm	85 (74.0)
T3: >5 cm	12 (10.4)
LN metastases	
Negative	38 (33.0)
Positive	
1-3 LN	18 (15.6)
>3 LN	59 (51.4)
Lymphovascular invasion	
Negative	32 (27.8)
Positive	83 (72.2)
Estrogen receptor status	
Negative	44 (38.2)
Positive	71 (61.8)
Progesterone receptor status	
Negative	43 (37.3)
Positive	72 (62.7)
Her-2/neu status	
Negative	71 (61.8)
Positive	44 (38.2)
Molecular subtypes	
Luminal A	54 (47.0)
Luminal B	32 (27.8)
Basal-like	21 (18.3)
Her2/neu	8 (6.9)

Her2 - human epidermal growth factor receptor 2

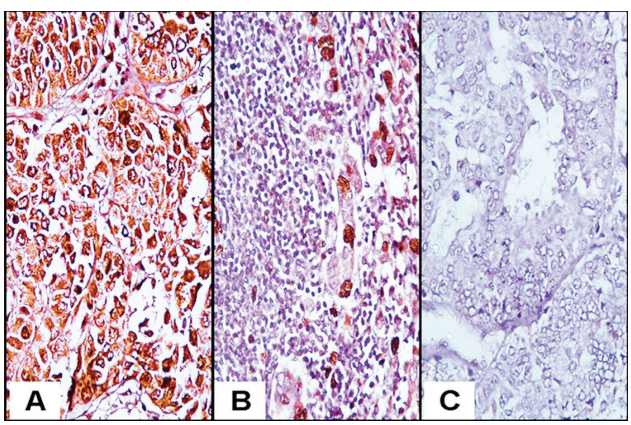

Figure 1 - Three tissue sections stained with anti-Ki67 antibody showing **A)** invasive duct carcinoma with high Ki67 PI (>90%), **B)** lymph node metastatic invasive duct carcinoma with high Ki67 PI (>90%), and **C)** invasive duct carcinoma with low Ki67 PI (<5%). Magnification x400

Table 2 - Ki67 proliferative index correlations with individual and combined immunoprofiles data and clinicopathological parameters of 115 breast cancer cases.

Parameters	Low Ki67 (n=30)	High Ki67 (n=85)	*P*-value
Age (years)			0.02
≤50	21	37	
>50	9	48	
Tumor grade			0.01
Grade I	3	3	
Grade II	20	38	
Grade III	7	44	
Tumor size (cm)			0.291
T1: <2	7	11	
T2: 2-5	19	66	
T3: >5	4	8	
Lymph node metastases			0.001
Negative	8	30	
1-3	11	7	
>3	11	48	
Lymphovascular invasion			0.689
Negative	7	25	
Positive	23	60	
Estrogen receptor status			0.377
Negative	14	30	
Positive	16	55	
Progesterone receptor status			0.149
Negative	15	28	
Positive	15	57	
Her2/neu status			0.009
Negative	25	46	
Positive	5	39	
Molecular subtypes			0.04
Luminal A	20	34	
Luminal B	5	27	
Basal-like	5	16	
Her2	0	8	

Her2 - human epidermal growth factor receptor 2

Discussion. Ki67 (anti-MIB1) has emerged as a rapid and inexpensive method to detect proliferation in BC. Despite the controversies regarding the value of Ki67, there is a strong data in the literature to show that ki67 is an excellent prognostic and predictive marker. In this study, the Ki67 PI in 115 Saudi BC patients from Almadinah Almonawwarah region was assessed using immunohistochemical staining. The standardized guidelines of the Breast Cancer Working Group for assessment of Ki67 had been followed, and the cut-off level used was 25%.[14,15] Although our study was a retrospective study, and it had the limitation of non-availability of all the historical demographic data and paraffin blocks; however, most BC patients were included. This study demonstrated a high Ki67 PI in most of the specimens (73.9%). This is consistent with the study from Libya[16,17] reporting high Ki67 PI in 76% patients. Furthermore, a group from Nigeria[18] have also reported a higher percentage (82.6%) of Ki67 PI in their patient cohort, whereas Altamimy et al[19] from KSA reported a significantly high prevalence of Ki67 PI (78.3%) in the basal class of BC. Our findings are in keeping with all these cited studies from Africa and middle east in contrast to western findings.[16-20] This may be because BC from Africa and Arab patients differ from those in BCs from western patients with regard to their presentation at an earlier age, metastatic potential, and reported aggressive behavior and mortality rates.[4,21] This high Ki67 PI was found to be significantly associated with bad prognostic clinicopathological parameters, such as old age, high grade tumors, and lymph node metastasis. This is consistent with findings of many previous studies[16-22] regarding the predictive and prognostic role of ki67 in BC. This confirms the biological principle in malignant neoplasms, when tumor cells proliferate at high rate become less able to differentiate.[23] A number of previous studies[22,24] have also reported positive association between high Ki67 PI and larger tumor sizes, and similarly with high Ki67 PI and hormonal status. In the present study, we did find some association of Ki67 PI with the above parameters; however, the association did not reach the level of statistical signifance. This may be because BC is a heterogeneous disease and sampling factor may have played a possible role at this point.[25] When we compared the Ki67 PI among different BC molecular phenotypes, we found that the Her2 group had the highest (100%) PI, followed by the luminal B (84%), then the basal group (76%). Whereas Ki67 PI was the least in luminal A type (62%). If we analyze the molecular subtypes, both Her2 group and luminal B types are Her2 positive, and Her2 is a well known oncoprotien. When Her2/neu is over expressed in BC, it stimulates cell proliferation.[26] Comparing the Ki67 PI in the 2 luminal groups, a significant difference between luminal-A group (62%) and luminal-B (84%) was detected in our study. This finding of high Ki67 PI in the luminal B group has also been confirmed by other studies,[27,28] this may be attributed to the Her2/neu positivity in the luminal B group.

In conclusion, in this study of Saudi patients, the Ki67 PI is significantly higher compared with rates reported in the western patients and were highest in the Her2 and luminal-B molecular subtypes. Ki67 PI can be an important predictive and prognostic marker in managing our Saudi BC patients. We recommend that it should be routinely performed on BC samples in diagnostic histopathology laboratories, following the guidelines proposed by the Breast Cancer Working Group, for better harmonization.

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