Ki-67 Expression in Breast Cancer, Its Correlation with ER, PR and Other Prognostic Factors in Nineveh Province

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ABSTRACT

Objectives: The aggressiveness of malignant tumors of breast can be correlated with the proliferation of neoplastic cells, and this detected by immunohistochemical study of proliferative index (Ki-67). American Society of Clinical Oncology (ASCO) does not recommend the use of Ki-67 routinely to predict the outcome of breast cancers, therefore the aim of current study is to detect the expression of Ki-67 in patients with primary breast cancer in Nineveh Province/North of Iraq and to correlate it with estrogen and progesterone receptors in addition to other prognostic factors.

Methods: In this retrospective-case series study eighty cases of histologically proven primary breast carcinomas were included. The cases were collected from hospitals and private laboratories in Nineveh Province / North of Iraq and studied Immunohistochemicaly for Ki-67, estrogen receptor (ER) and progesterone receptor (PR) were done on tissue sections embedded in paraffin wax. An area with the maximum proliferation was chosen to evaluate Ki-67 and the cases with ≥20% positive nuclei were considered as high Ki-67 expression while those with <20% positive nuclei were considered as low Ki-67 expression. The findings of Ki-67 were correlated with the age of the patients, histological type, grade of the tumors and with the estrogen and progesterone receptors.

Results: The Ki-67 immunoreactivity was highly expressed in (45%) of the cases. Estrogen and progesterone receptors observed in (77.5%) and (67.5%) of the cases respectively. The Ki-67 was significantly associated with grade of tumor, estrogen receptor and progesterone receptor (P= 0.0057, 0.037 and 0.006 respectively). While the association with patients age and histological types were not statistically significant.

Conclusion: Ki-67 expression shows a significant direct correlation with grade of tumors and a significant inverse correlation has been shown with a well-known predictive factors, (estrogen and progesterone receptors).

Key words: breast cancer, immunohistochemistry, Ki-67, estrogen receptor and progesterone receptor.

تعبير Ki-67في سرطان الثدي وعلاقته مع مستقبلات الاستروجين و البروجستيرون و العوامل النذيرية الاخرى في محافظة نينوى

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الخلاصة

الهدف : إن عدوانية سرطان الثدي ترتبط بتكاثر الخلايا السرطانية والتي يمكن تحديدها بطريقة كيميائية نسيجية مناعية باستخدام مؤشر التكاثرKi-67 .استخدام Ki-67 لتحديد النذير المستقبلي لحالات سرطان الثدي لم ينصح بإستخدامه كروتين من قبل الجمعية الامريكية لعلم الاورام السريرية(ASCO),لهذا فإن الهدف من هذه الدراسة تقييم تعبير ال Ki-67في سرطانات الثدي الابتدائية في محافظة نينوى/شمال العراق وايجاد علاقته مع مستقبلات الاستروجين والبروجيستيرون والعوامل النذيرية الاخرى.

الطريقة : ثمانون حالة من حالات سرطان الثدي الابتدائية المشخصة نسيجياً تم ادخالها في هذه الدراسة وقد جمعت هذه الحالات من المستشفيات والعيادات الخاصة في محافظة نينوى/شمال العراق، تم دراسةKi-67 ومستقبلات الاستروجين والبروجستيرون

بطريقة كيميائية نسيجية مناعية على المقطع النسيجي لكل حالة والمغمورة في شمع البارافين. نتائج تعبير Ki-67 قيمت مع عمر المرضى والنوع النسيجي للورم ودرجة الورم و مستقبلات الاستروجين والبروجيستيرون.

النتائج : لقد كان متوسط عمر المرضى50.4 سنة وسرطان الاقنية غير المحدد كان اكثر الانواع النسيجية وشكل 86.25% من الحالات وأن 82,6% من هذه الحالات كانت من الدرجة العالية(درجة I وII). تعبيرKi-67 كان عالي (اكثر أو يساوي 20% من الخلايا السرطانية موجبة) في 45% من الحالات و مستقبلات الاستروجين والبروجيستيرون كانت موجبة في 77.5% و 67.5% من الحالات على الترتيب و لقد اظهرKi-67 علاقة معنوية مع درجة الورم ومستقبلات الاستروجين والبروجستيرون (P=0,0057 و0,037 و0,006) بينما لم يظهر اي علاقة مع عمر المرضى والنوع النسيجي للورم.

الاستنتاجات : في هذه الدراسة, أظهر تعبير Ki-67 علاقة معنوية مباشرة مع درجة الورم وعلاقة عكسية معنوية مع العوامل التنبوئية المعروفة (مستقبلات الاستروجين والبروجستيرون). نحتاج الى دراسة اخرى مع متابعة لحالات مرض سرطان الثدي والتي يجب أن تضمن مختلف تعبير Ki-67 ومختلف مستقبلات الاستروجين والبروجستيرون لاثبات التأثير النذيري والتنبؤي لل Ki-67.

الكلمات المفتاحية : سرطان الثدي, تعبير ال Ki-67, مستقبلات الاستروجين والبروجستيرون.

INTRODUCTION

Worldwide, the most common malignant tumor in women is the breast cancer (BC) with an annually new diagnosed cases of more than 1.7 million patients and this number is increasing and may reach up to a 3.2 million per year by 2030.[1]. Since three decades in Iraq, BC is ranking the first among the Iraqi population.[2], in female BC account up to 29% of all newly diagnosed cancer and it is responsible for cancer related deaths in 14% of the cases.[3]. Better outcomes of breast cancer are associated with the expression of ER and PR receptors.[4] , they act as predictors of a good response to hormonal (anti-estrogen) therapy. Proliferation rates can give useful information on aggressiveness and prognosis of cancers, also can be used as a guide for treatment regimen in clinical practice.[5] The Ki-67 has emerged as an easy, rapid and economically suitable marker to detect proliferation rate in BC.[6] .

"Ki-67 is a non-histone nuclear cortex protein.[7,8], involved in the early steps of polymerase I-dependent ribosomal RNA synthesis. It was first identified in a Hodgkin lymphoma in 1983".[8]. "It is expressed in all continuously cycling cells of mid-G1, S, and G2 phase and in mitosis, but not in the G0 and early G1 phase".[7,9,10]. Ki-67 plays an important role in cell proliferation, but its precise function is still unknown. [9] So this research is performed on the paraffin embedded blocks of patients with primary breast cancer in Nineveh Province to assess Ki-67 expression, to correlate the Ki-67 findings with patients' age, histological types, grade of tumors , with ER and PR receptors expression, and to detect any significant differences in the current results as compared with that of other studies.

PATIENTS AND METHODS

This study was reviewed and approved by Medical Research Ethics Committee (MREC), College of Medicine, University of Mosul. In this retrospective-case series study, a formalin-fixed paraffin embedded tissue blocks of an eighty female patients with primary breast cancer were collected from General hospitals and private laboratories in Nineveh province at the north of Iraq during the period from first of October 2017 to first of August 2018. Sections from paraffin embedded tissue which H&E stained, re-reviewed under light microscope to assess the primary tumor ,the histological types were determined by WHO classification of breast tumors and graded by Modified Bloom-Richardson grading system. The age of the patients was retrieved from the medical reports. One section from each case was selected for immune-histochemical study.

Immunohistochemistry

Each case was studied for ER, PR and Ki-67. The antibodies, buffers, glass slides and linking systems were purchased from DAKO™ (Dako/Denmark). Sections of 4 millimeter thickness were deparaffinized in xylene and dehydrated, the immunohistochemical study was performed according to manufacturer instructions. Primary

antibodies used for ER was (1D5 Dako Cytomation, dilution 1:100), for PR was (PgR636 Dako Cytomation, dilution 1:100) and for Ki-67 was (MIB-1 (M7240; Dako Cytomationdilution, dilution 1:100)). With each run a positive and negative controls were included. Negative controls were prepared by replacing the primary antibody with Tris-buffered saline, positive control slides for ER and PR were obtained from breast carcinoma known to be positive for the hormone receptors while for Ki-67 a sections from tonsils were used as positive controls.

Immunohistochemical Evaluation

The immunostaining of ER, PR and Ki-67 appear as brown nuclear stain. All slides were evaluated for this immune-staining by light microscopy using a Leitz dialux microscope. Regarding the ER and PR expression the cases were considered positive if ≥10% of the neoplastic cells showed positive nuclear staining.[10]. Ki-67 was expressed as a percentage of positively stained cells per 100 epithelial cells after counting at least 1000 cells using high power (400X). An area with the maximum proliferation was chosen to evaluate Ki-67 and the cases with ≥20% positive nuclei were considered as high Ki-67 expression while those with <20% positive nuclei were considered as low Ki-67 expression.[10-14].

Statistical Analysis

The association between Ki-67 and variable categories was assessed using Chi-square test and Fisher Exact test when indicated. Regarding statistical analyses, the P value considered statistically significant if its < 0.05.

RESULTS

The patients' age ranged from 24 – 74 years (mean =50.4) with 43 cases (53.75%) were younger than 50 years. Regarding the histological types of the cases were included in the study; the majority of cases (69 out of 80) were of IDC-NOS, which form 86.25% of all the cases, the remaining cases were: 8 cases invasive lobular carcinoma (ILC) which form 10% and 3 cases were ductal carcinoma in situ (DCIS) which form 3.75% of all cases. Concerning the grading of IDC-NOS that were included in this study; 12 cases (17.4%) were of grade I, grade II were 25 cases (36.2%) and 32 cases (46.4%) were of grade III. The ER was found to be positive in 62 cases (77.5%) while PR was positive in 54 cases (67.5%), [Figure 1]. Immunohistochemical study of Ki-67 showed highly expression (≥20% of nuclei were positive) in 36 (45%) of patients and low expression was observed in 44 cases (55%), [Figure 2]. The patients' characteristics are summarized in table(1).

The correlation of Ki-67 expression with the age of the patients and the histological types were not significant statistically (P value 0.333 and 0.682 respectively). A significant direct association of Ki-67 expression with the grade was observed (P=0.0057) while inverse relation was found between Ki-67 expression and ER positivity that is a high Ki-67 expression was associated with decreased ER positivity, this association was statistically significant (P=0.037). The same trend was found with PR positivity and also the relation was statistically significant (P=0.006).

Regarding the hormonal status, more than half of the patients (53 cases which form 66.25%) were positive for both ER and PR (ER+PR+), 36 cases (45%) of them showed low Ki-67 expression, 9 cases (11.25%) were ER+PR-, 7 cases of them had a high Ki-67 expression, only one case (1.25%) was ER-PR+ and it showed a high Ki-67 expression, while 17 cases (21.25%) were ER-PR- and 11 cases of them showed a high Ki-67. This inverse relation of Ki-67 with hormonal status was statistically significant (P=0.0054). Tables (2 and 3) summarized the relation of Ki-67 with various parameters and with histological types respectively.

DISCUSSION

The age of patients included in this study were ranged from 24 to 74 years (mean = 50.4), this result is very near to that of another Iraqi study done in 2016 in Baghdad by Al-Rawaq [15] , in which the mean of age was 51 with the range of 27-73, also it somewhat nears to another study done in Nineveh Province by Al –Nuaimy [16] , in 2015. However the result is more or less within those of others. [4,6,9,11,12,17,18,19], this may be due to genetic difference, hereditary factors, socio-demographic factors, the age of menarche, parity and time of first delivery in addition to the close relative

marriages which are common in Iraq. Obesity and Lack of exercise which have been newly prevalent in Iraq can be added.

Forty three (53.75%) of the cases included in this study were less than 50 years old while 46.25% were 50 years or older, this result is opposite to what is known about the increasing incidence of BC with age, however this result is similar to that recorded by other Iraqi studies, that 54.7% and 58% of the cases included in a studies done by Al - Nuaimy [16] , and Mahmoud [20] respectively, were younger than 50 years. This also may be due to socio-demographic factors, genetic difference, age of menarche, time of first delivery, parity, traditional marriages among first-degree relatives [16]. In addition may be attributed to effect of the wars that Iraq was exposed, that make Iraqi people exposed to a lot of chemical carcinogenic material and radiation. More researches are needed in this locality to determine the predisposing factors for this result.

In this study, the IDC-NOS was the most common histological type which form 86.25% of the cases, and this result is near to that found by Neelakanth [21] , where 90.7% of the cases were IDC-NOS, the result also somewhat near to that of Mahmoud [(20)] وwho observed that 93.5% of the cases included his study were of IDC-NOS and also it was the most common in other studies [4,9,10,13,16]

Regarding the histological grade of tumor, the maximum number of IDC-NOS cases (82.6%) that included in this study were presented with high grade (grade II and III), with 46.4% of them were of grade III, this result is similar to that of a study done in Kirkuk/Iraq in 2014 by Mahmoud [20] in which grade III was formed 46.4% of his cases. Also Grade III was common (53% of the cases) in a study done by Soliman [9] in Egypt. However this result is differ from that observed by others. [4,12,16] where grade II was the most common, this may be attributed to difference in demographic characteristics, racial backgrounds or probably reflecting tumor cell heterogeneity.

In the current study, a positive immunohistochemical ER and PR were observed in 77.5% and 67.5% respectively, these results were near to that study done in Morroco in 2016 by Mahir W et al. [19] which showed that ER and PR were positive in 73.1% and 69.1% respectively, Furthermore, the result of this study also relatively near to that of a study done in Baghdad in 2015 by Al-Sarraf [4] in which 75% and 72.5% of the cases showed a positivity for ER and PR respectively and consistent with other study done also in Baghdad in 2012 by Elyass [22] , where ER and PR were positive in 72% and 68% of the cases respectively, However different results were seen by others.

Table (4). The variation in the findings of these studies may be due to variation in the IHC staining technical operation with different manufacturers, variation of kits and types of antibodies which were used in these studies in addition to the variation of patients' age group.

The Expression of Ki-67 in This Study and Its Relation with Various Parameters

"Ki-67 antigen was detected in the early steps of polymerase I-dependent ribosomal RNA synthesis".[14]. Despite Ki-67 protein has a significant role in cell proliferation , but its precise function is still unknown and there is few published researches on its role.[14]. A number of researches have studied the prognostic significance of Ki-67 in breast cancer. But no similar study was done in Nineveh province. So the current study was also done to evaluate the prognostic significance of Ki-67 in primary breast cancer by correlating it with traditional well known clinicopathological prognostic parameters like age of the patients, histological types, grade of tumor, and its correlation with the ER and PR which are most useful markers in predicting response to hormonal therapy.

Despite the presence of a large number of published researches reporting a significant results regarding the Ki-67, but still the cut-point for differentiate a BC patients with low Ki-67 expression from a high expression is currently debatable [23,24], which was varied from 1 to 29% and this may be the cause for limiting its clinical utility.[24]. The St. Gallen Consensus in 2009, classify Ki-67 expression into: low<15%, intermediate 16–30% and high>30. [24,25]. Then St. Gallen in 2011 proposed Ki-67 cut-point at 14% and use it to separate ER + tumors into luminal A

(Ki-67<14%) and luminal B (Ki-67>14%).[13,24], but 2 years later, it was upgraded "to 20% with the option to also use locally specified cut-points".[13,26], then St. Gallen Breast Cancer Conference in 2015 has been recommended a median cut-off value of Ki-67 within the range of 20–29% in order to define 'luminal B-like'.[10]. In 2016 a study was done by Bustreo et al. [13] on 1.577 HER2-/ER+ breast cancer patients having full clinical, histolopathological, and follow-up data, and they use two Ki-67 cut-offs (14 and 20 %) and correlated them with disease-free interval (DFI) and disease-specific survival (DSS). They found that patients having tumors with Ki-67<14 % did not differ from those with Ki-67 values between 14 and 20 % regarding DFI and DSS, while the poorest prognosis was observed in those patients having tumor with Ki-67 >20%. In the current study, the 20% as a cutoff value for Ki-67 was used, also this cut-point was used by other studies.[10-14].

In this study, the high Ki-67 expression was observed in 36 cases (45%). This is consistent with that study done by Madani [18] in Iran in 2016 in which a high expression of Ki67 were observed in 44.6% of the cases and also the result near to that found by Joensuu K.[7] in Finland were 43% of the cases showed a high Ki-67 expression, however the result of this study is differ from that of others, see table (4).

The commonest way to study Ki-67 expression is immunohistochemical (IHC) staining by MIB-1 antibody. Different antibodies such as Ki-S5, MIB-1, MM-1, and SP-6 were used in different studies. [8,14,25], this might be a reason for this considerable variation. Also these variations may be attributed to different groups of population, different methods for detecting Ki-67 and different cutoffs to differentiate a high Ki-67 expression from a low. [27]. In addition to that there is variation in Ki-67 scoring that, some of the pathologists use the percentage of nuclei staining; while others enumerate several hundred nuclei in different areas of tumors to get an overall average index. [25].

The relation of Ki-67 expression with the age of the patients was not statistically significant (P=0.333), this result is similar to that of others.[9,23], however the Liu et al.[12] found a significant relation of Ki-67 with the age, that patients with higher Ki-67 expression were younger than those with lower Ki-67 expression. No significant relation was found between Ki-67 and histological types, while Neelakanth [21] found a significant relation with histological type, relatively small numbers of non-ductal types included in this study may be the cause of this result, however Soleman [9] and Ermiah et al.[23] also found no significant relation between Ki-67 and the tumor histological type.

A definite significant correlation between the Ki-67 expression and tumor grade has been documented in several studies.[5,6,9,12,21,23,26,27], a similar result was observed in the current study, this is due to that mitotic index is one of the three components of the Modified Bloom-Richardson grading system of BC.[21]. It is well known that histologic grade has been associated with poor prognosis. [21] In current study Ki-67 expression was found increase with increasing the grade of tumor. This goes with the well-established view, that proliferating cells usually do not differentiate while cells when differentiating, usually stop dividing.[21] .

Regarding the hormonal status, most of the breast cancer patients (66.25% of the cases) in the current study were hormonal receptors positive (ER+PR+), this result is similar to that of Al Sarraf [4] and Al-Rawaq [15]. Hormonal receptors, which are well known predictive and prognostic biomarkers, were used to determine benefit from endocrine therapies in BC. A significant inverse relation was observed between Ki-67 and ER and between Ki-67 and PR that is patients with ER- and / or PR- are more possibility to have a higher Ki-67 expression, this result was also observed by others. [5,9,12,23,26,28] Liu et al.[12] in their study were concluded that the BC with high Ki-67 expression has a poor prognosis but responds better to neoadjuvant chemotherapy, this may be due to the fact that dividing cells have increased sensitivity to cytotoxic drugs [29]. Faneyte et al.[30] observed that Ki-67 was significantly declined after chemotherapy, also they observed that a patients with BC that have a negative ER and a high Ki-67 expression are associated with better response. [30].

So in the BC, Ki-67 expression may be considered as a predictive marker that can be added to ER and PR to predict the prognosis. A large prospective study of DFS and OS among BC

patients with various Ki-67 expression and various ER/PR cancer phenotypes is needed to determine the prognostic and predictive effect of Ki-67.

CONCLUSION

the current study a high Ki-67 expression was found in 45% of a female with BC in Nineveh Province /north of Iraq. No significant relation was found among Ki-67 with the age of patients and histological types of BC, A direct significant relation was observed between the Ki-67 expression and grade of tumor while Ki-67 was inversely associated with a well-known predictive factors (ER and PR). Further study with follow-up of the BC patients with various Ki-67 expression and various ER/PR cancer phenotypes is required to improve the prognostic and predictive role of Ki-67 in BC.

RECOMMENDATION

- More studies are advised in our locality to detect the predisposing factors for occurrence of BC in younger age groups among Iraqi population.
- The Ki-67 is an immunohistochemical marker which determines the proliferative activity of tumor, so can give an idea about the aggressiveness of tumor and the patients prognosis.
-The Ki-67 can be used to make a subclassification of the patients, and can be used with other immunohistochemical markers (ER and PR) for better classification of patients, to include them under appropriate treatment regimens and to avoid them the ineffective therapy.
-Further study with follow-up of the patients with breast cancer with various Ki-67 expression and various ER/PR cancer phenotypes is required to prove the predictive and prognostic effect of this marker.

Table (1): The data of the patients included in this study

		No.	%
Age	<50	43	53.75%
	≥50	37	46.25%
Histological type	IDC-NOS	69	86.25%
	ILC	8	10%
	DCIS	3	3.75%
Grade of IDC-NOS	I	12	17.4%
	II	25	36.2%
	III	32	46.4%
Estrogen receptor	Positive	62	77.5%
	Negative	18	22.5%
Progesterone receptor	Positive	54	67.5%
	Negative	26	32.5%
Hormonal receptors status	ER+PR+	53	66.25%
	ER+PR-	9	11.25%
	ER-PR+	1	1.25%
	ER-PR-	17	21.25%
Ki-67 expression	High	36	45%
	Low	44	55%
Total no. of the cases		80	100%

Table (2): Relation of Ki-67 expression with the different parameters

Parameters		Ki-67		Total	P-value
		High expression	Low expression		
Age of patients	<50 year	22(27.5%)	21(26.25%)	43(53.75%)	0.333
	≥50 year	14(17.5%)	23(28.75%)	37(46.25%)	
Grade of tumor	Grade I	2(2. 9%)	10(14.5%)	12(17.4%)	0.0057
	Grade II	18(26.1%)	7(10.1%)	25(36.2%)	
	Grade III	16(23.2%)	16(23.2%)	32(46.4%)	
ER	Positive	23(28.75%)	38(47.5%)	61(76.25%)	0.037
	Negative	13(16.25%)	6(7.5%)	19 (23.75%)	
PR	Positive	18(22.5%)	36(45%)	54(67.5%)	0.006
	Negative	18(22.5%)	8(10%)	26(32.5%)	
Hormonal receptors (ER and PR) status	ER+PR+	17(21.25%)	36(45%)	53(66.25%)	0.0054
	ER+PR-	7(8.75%)	2(2.5%)	9(11.25%)	
	ER-PR+	1(1.25%)	0(0%)	1(1.25%)	
	ER-PR-	11(13.75%)	6(7.5%)	17(21.25%)	

Table(3):Relation of Ki-67 with histological type of tumors.

Histological types	Ki-67 expression		Total	P value
	high	low		
IDC-NOS	30(37.5%)	39(48.75%)	69 (86.25%)	0.682
ILC	4(5%)	4(5%)	8 (10%)	
DCIS	2(2.5%)	1(1.25%)	3 (3.75%)	
Total	36(45%)	44(55%)	80(100%)	

Table(4): The number and percentage of high Ki-67 expression and the percentage of positive ER and PR in the breast cancer cases included in the current study and other studies.

Studies	Year	Region	High Ki-67 No. (%)	ER%	PR%	Total No. of cases
Current study	2018	Nineveh/Iraq	36 (45%)	77.5%	67.5%	80
Liu Z et al. [12]	2017	China	249 (62.6%)	56.5%	18.1%	398
Kombak FE et al. [17]	2017	Turkey	154(65.2%)	82.7%	75.7%	236
Elkablawy MA. [6]	2016	Kingdom of Saudi Arabia	85 (73.9%)	61.8%	62.7%	115
Soliman NA. [9]	2016	Egypt	36(33.8%)	53.3%	55.1%	107
Al-Rawaq KJ. [15]	2016	Baghdad/Iraq	69 (69%)	74%	75%	100
Madani SH. [18]	2016	Iran	116(44.6%)	60%	57.3%	260
Al-Sarraf FS. [4]	2015	Baghdad/Iraq	23(57.5%)	75%	72.5%	40
Joensuu K. [7]	2013	Finland	31(43%)	68%	53%	72
Ermiah E et al. [23]	2012	Libya	76(76%)	67%	55%	100

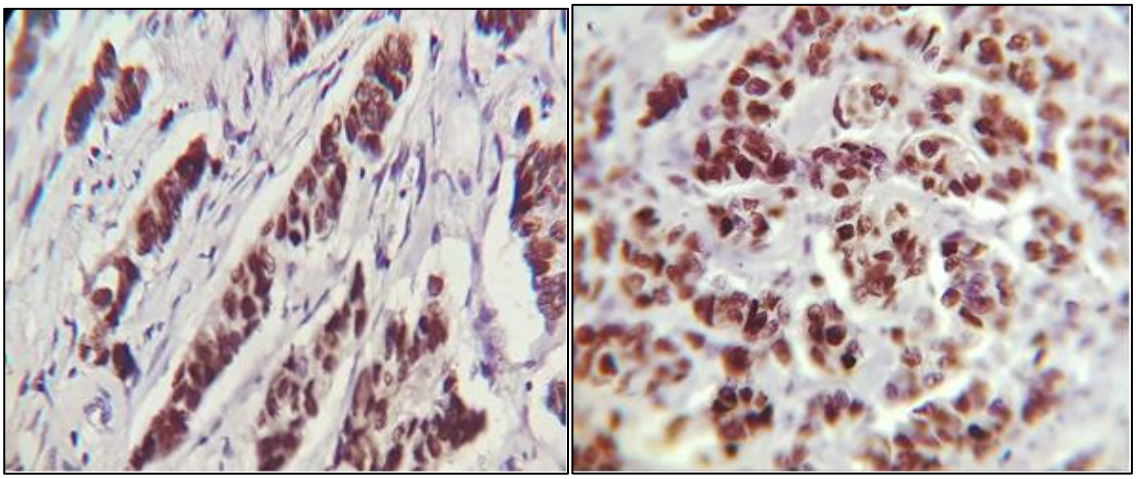

Figure (1): Breast cancer positive for hormone receptors. Left, positive for ER and right, positive for PR (X400).

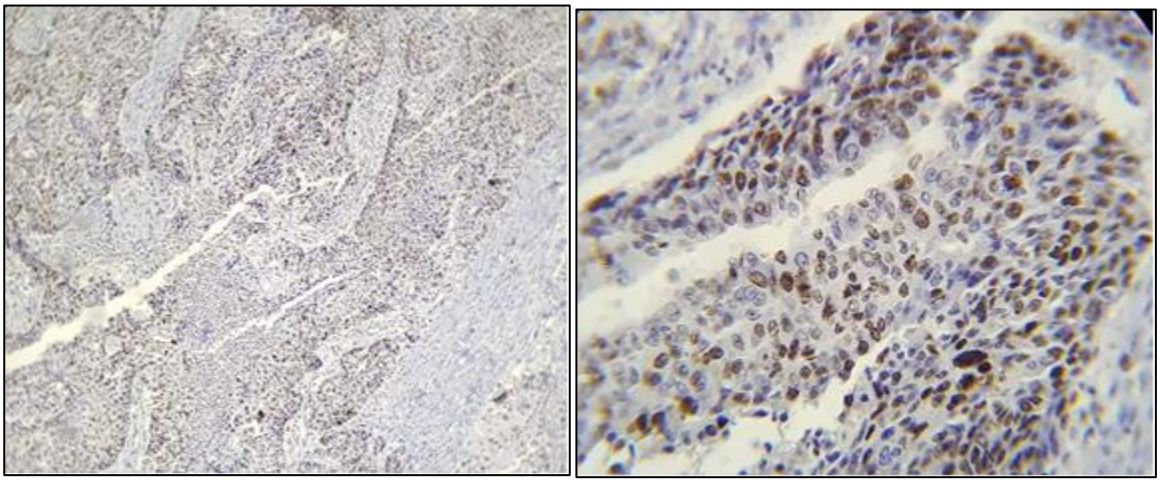

Figure (2): High Ki-67 expression in breast cancer (left X40 and right X400)

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