Original Article

Evaluation of the proliferation marker Ki67 as a prognostic factor in patients with breast carcinoma

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Abstract:

Background: Breast cancer is the most frequent cancer in women worldwide and in Iraq. Proliferation rates of neoplastic process can be useful in predicting prognosis, aggressiveness of cancers and to guide treatment protocols in clinical practice.

Objectives: To evaluate the role of Ki67 as a proliferative marker through analysing the associations between Ki67 with the clinic-pathological parameters, hormone receptors and Her2/neu expression.

Patients and methods: Forty paraffin blocks belonging to patient with breast carcinoma and ten blocks with benign diseases were included in this retrospective cross-sectional study and used for the immunohistochemical assessment of hormone receptors, Her2/neu and Ki67.

Results: Mean age of the malignant cases was (50.30±9) years; invasive ductal carcinoma was the main histopathological type (87.5%). Three quarters of the cases were with (Grade II) and (T2). Positive lymph node reported in (72.5%) of cases. Malignant cases positively expressed ER, PR, Her2/neu (score 3+) and Ki67 in (75%), (72.5%), (17.5%) and (75%) respectively. Luminal B subtype was the commonest among studied cases.

Conclusions: Ki67 proliferative index represented a valuable tool and provided information about aggressiveness and prognosis of breast carcinoma, significant correlations found between Ki67 and tumor grade, lymph node involvement and Her2/neu score.

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Introduction:

Breast carcinoma is the most common malignant tumour and the leading cause of carcinoma death in women, with more than 1,000,000 cases occurring worldwide annually.[1] In Iraq, breast cancer is the most common type of malignancy among the Iraqi population in general. It accounts for approximately one third of the registered female cancers according to the latest Iraqi Cancer Registry. [2] The important prognostic indicators in histopathology are tumour size and extent, histopathological type, grade and lymph node status. In addition, other factors which are not only predictive of outcome, but also have a role in predicting therapy response against particular molecular targets.[3] Some of these factors are:

Hormone receptors (ER, PR): There presences are correlated with a better outcome and they are important predictors of response to hormonal (anti-oestrogen) therapy. Her2/neu overexpression is associated with poorer survival, but it's important in predicting response to agents that target this trans-membrane protein (e.g. Trastuzumab or Herceptin).

Proliferative rate: In addition to mitotic counts, proliferation can be measured by immunohistochemical detection of cellular proteins produced during the cell cycle, for example:

Ki67. Carcinomas with high proliferation rates have a poorer prognosis but may respond better to chemotherapy. [3]Ki67 nuclear antigen is expressed in certain phases of the cell cycle S, G_1, G_2, and M phases, but not in G_0. [4]Studies of breast cancers using gene expression profiling have identified several major breast cancer subtypes. The most reproducibly identified molecular subtypes are luminal A, luminal B, Her2 subtype and basal-like group or triple negative. [5] These breast cancer molecular subtypes differ with regard to their patterns of gene expression, clinical features, response to treatment, and prognosis.[6]

Patients and methods:

This retrospective cross-sectional study was conducted from Nov. 2013 to April 2014. A total number of fifty (50) blocks of breast tissue were diagnosed in a period from June 2012 to March 2014 were collected, all belonged to female patients. The blocks of the selected cases included (40) biopsies (all were total mastectomy) diagnosed as breast carcinoma (invasive ductal carcinoma (35) cases, invasive lobular carcinoma (4) cases and mixed type carcinoma (1) case), and ten (10) biopsies of benign breast diseases (fibroadenoma (4) cases, fibrocystic diseases (6) cases). The cases were selected from archive files of the Department of Pathology of the Teaching Laboratories and private laboratories. Clinic-pathological information including the age of the patients, histological type, malignant

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tumor grade, size, as well as lymph node involvement were obtained from reviewing the available reports. For each case, five sections of (4 µm) thickness were prepared, one section stained with H&E stain for histopathological diagnosis, others stained immunohistochemically for ER, PR, Her2/neu and Ki67, which were performed by LSAB method (Labelled Streptavidin-Biotin peroxidase) on automated immunohistochemistry stainer (Denmark) using detection reagents and buffers supplied by the manufacturer (Dako). Negative control slides were obtained by replacing the primary antibody with Tris-buffer saline for markers while Positive controls slides were obtained by using breast cancer sections that were known to be immunoreactive for ER, PR and Her2/neu and pharyngeal tonsillar hyperplasia sections were stained for Ki67, sections with strong and high Ki67 expression served as positive control.Evaluation of the results and scoring: slides were scanned by X10 magnification and five cellular areas selected (i.e. hot spots) and evaluated at X40 magnification, the criteria for positive immunoreactions are any brown nuclear staining for ER and PR, dark brown precipitate at the cell membrane for Her2/neu protein and Ki67 immunoreactivity was considered positive, irrespective of stain intensity.ER and PR scoring was performed according to the Allred Score System (Allred et al., 1998) [7] which includes proportion score (PS) and intensity score (IS) to get the total score. Her2/neu was scored on a (0) to (3) scale according to the criteria set by Dako. The staining was scored as: negative (0) when no membrane staining was observed, or when membranous staining was observed in (<10%) of the tumor cells; weak positive (1+) if weak focal membrane staining was seen in (>10%) of the tumor cells; intermediate (2+) if weak to moderate, complete membrane staining was seen in (>10%) of the tumor cells; and strongly positive (3+) if intense membrane staining with weak to moderate cytoplasmic reactivity was seen (>10%) of the tumor cells. In the final analysis, scores 0 and 1 were considered negative; score 2+ was considered weakly positive; and score 3+ was considered strongly positive. Only score 3+ cases were considered as Her2 over expressing cases. Ki67 proliferative index was determined and defined as low when the number of Ki67 positive cells was (< 14%), and high if the number of Ki67 positive tumor cells was (≥ 14%).

Statistical Analysis: Data were analyzed using SPSS20 and Microsoft Office Excel 2010. Analysis of the significance of difference of different percentages was tested using Pearson Chi-square test (X^2-test) while t-test was used to analyse numeric data. Statistical significance was considered whenever the P value was less than 0.05.

Results:

In this study, the mean age of malignant cases was (50.30±9) years with a range of (34-69) years, most of the cases belonged to the (35-50) years age group. The majority of the malignant cases were hormone receptors positive, Her2/neu negative and Ki67 positive with high score. As shown in table 1.

Table 1 Distribution of malignant cases according to clinic-pathological parameters.

Main category	Subcategories	No.	%
Histopathological type	Invasive ductal carcinoma IDC	35	87.5%
	Invasive lobular carcinoma ILC	4	10%
	Mixed type	1	2.5%
Grade	grade I	2	5%
	grade II	30	75%
	grade III	8	20%
Tumor Size	T1	2	5%
	T2	30	75%
	T3	5	12.5%
	T4	3	7.5%
Lymph Node Involvement	Positive	29	72.5%
	Negative	11	27.5%
Estrogen receptor	Positive	30	75%
	Negative	10	25%
Progesterone receptor	Positive	29	72.5%
	Negative	11	27.5%
Her2/neu score	Score 0	21	52.5%
	1+	7	17.5%
	2+	5	12.5%
	3+	7	17.5%
Ki 67 expression	Positive	30	75%
	Negative	10	25%
Ki67 score	< 14	17	42.5%
	≥ 14	23	57.5%
Tumor subtypes	Luminal A	9	22.5%
	Luminal B	17	42.5%
	Her2	3	7.5%
	Triple negative	6	15%

The mean age of benign cases was (36.5±13.75) years old with a range of (22-60) years old. Fibrocystic diseases were the commonest histopathological type, all the cases were ER negative, Her2/neu negative and Ki67 positive (low score), and (60%) were PR positive. There were statistical significant differences between malignant cases and benign cases regarding ER expression, Her2/neu and Ki67 score.

Significant correlations were found between Ki67 and tumor grade, lymph node involvement and Her2/neu score. As shown in table 2.

Table 2 correlations of Ki67 with clinic-pathological parameters.

Main categories	Subcategories	Ki67 (≥14%) No.	%	Ki67 (<14%) No.	%	Total No.	%	P value
Age	< 35 years	2	5%	0	0%	2	5%	0.66
	35-50 years	12	30%	9	22.5%	21	52.5%	
	>50 years	9	22.5%	8	20%	17	42.5%	
Histopathological type	IDC*	19	47.4%	16	40%	35	87.5%	0.78
	ILC**	3	7.5%	1	2.5%	4	10%	
	Mixed type	1	2.5%	0	0%	1	2.5%	
Grade	grade I	0	0%	2	5%	2	5%	0.004
	grade II	15	37.5%	15	37.5%	30	75%	
	grade III	8	20%	0	0%	8	20%	
Tumor Size	T1	1	2.5%	1	2.5%	2	5%	0.92
	T2	18	45%	12	30%	30	75%	
	T3	2	5%	3	7.5%	5	12.5%	
	T4	2	5%	1	2.5%	3	7.5%	
Lymph Node Involvement	Positive	22	55%	7	17.5	29	72.5%	0.0001
	Negative	1	2.5%	10	25%	11	27.5%	
ER	Positive	19	47.5%	11	27.5%	30	75%	0.27
	Negative	4	10%	6	15%	10	10%	
PR	Positive	15	37.5%	14	35%	29	72.5%	0.29
	Negative	8	20%	3	7.5%	11	27.5%	
Her2/neu score	Score 0	12	30%	9	22.5%	21	52.5%	0.006
	1+	1	2.5%	6	15%	7	17.5%	
	2+	4	10%	1	2.5%	5	12.5%	
	3+	6	15%	1	2.5%	7	17.5%	

*Invasive ductal carcinoma. ** Invasive lobular carcinoma.

Also There was a statistical significant correlation between Her2/neu expression (score 3+) in the malignant cases and positive lymph node. As shown in fig. 1.

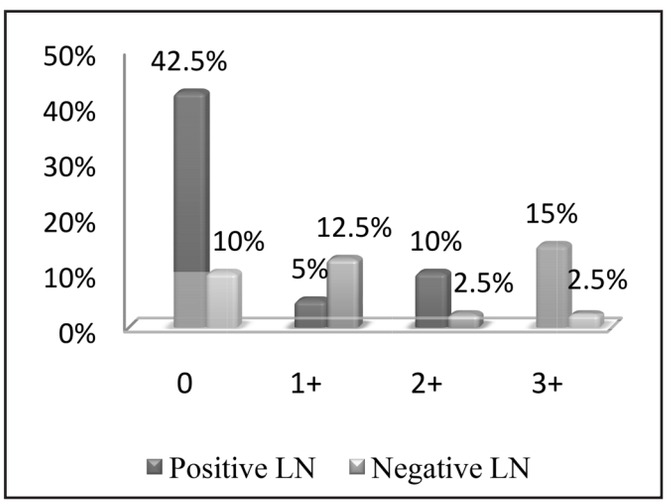

Fig. 1 Correlation of Her2/neu and lymph node involvement.

Discussion:

In this study the age range of the malignant cases was very similar to another Iraqi study [8] that reported age range of (33-69) and the mean age was comparable to that reported by the same study and a study from Lebanon [9] that had recorded a mean age of (48.5 and 49.8) years respectively; however the result of this study was lower than that recorded in the Western countries by a decade as reported by a study [10]. While in the benign cases, the mean age and age range were in agreement with a study from Europe [11] which showed a mean age of (37.12±10.12) with age range of (19-60) years. The age difference between malignant and benign cases is statistically significant (benign tumor patients were of younger age), this finding agreed with an Iraqi study. [12] In benign cases, fibrocystic diseases were (60%) and fibroadenoma were (40%) of cases. This disagreed with another study by Al-Alwan [13] who reported that fibroadenoma is more common than fibrocystic diseases, the difference may be due to the small sample size of the current study. Ki67 expression was demonstrated in (75%) of malignant cases with (9-80%) score range, and (57.5%) of malignant cases were with high score. This result was close to another study [14]. However, the recorded expression was higher than that reported by studies from Iraq [15] and Japan [16] which reported that Ki67 expression were in (38.5%) and (65%) of the cases respectively. All grade III cases were with Ki67 high score while all grade I cases were with Ki67 low score, a significant

correlation found between Ki67 and tumor grade, same results were observed by previous studies from Iraq [8] and Japan [16], while another study [17] showed insignificant correlation. There was statistical significant correlation between Ki67 and positive lymph nodes, this agreed with the following study [18], while disagreed with another study [17]. There was significant correlation between Ki67 score and Her2/neu score, this agreed with a study [19]. There was insignificant correlation between Ki67 and age of the patients this agreed with previous Iraqi study [15]. There was insignificant correlation between Ki67 and size of the tumor this agreed with a study [19] and disagreed with a study [8] that found significant correlation with tumor size. Also insignificant correlations were found between Ki67 and hormone receptors this was in agreement with a Pakistani study [19], but disagreed with an Iranian study [20] that showed significant correlation with PR but not ER. For Her2/neu, this study demonstrated that (17.5%) of malignant cases were Her2/neu positive (score 3+) and (70%) of cases were Her2/neu negative (score 0 and score 1+) this agreed with Al-Bedairy et al. Iraqi study in (2014) [21]. There was significant correlation between Her2/neu and lymph node involvement, this agreed with an Iraqi study [22] but disagreed with a study [23]. Insignificant correlation was found between Her2/neu and age of the patients, this agreed with other study. [22] while an Iraqi study [21] showed a significant difference. And insignificant correlation was found between Her2/neu and tumor type, this agreed with Mohmoud study [24] but disagreed with Arpino et al. study in (2004) [25], the correlation between Her2/neu overexpression and the tumor grades was insignificant, this agreed with a study [26] but disagreed with another [27]. The correlation between Her2/neu and tumor size was statistically insignificant, this agreed with an Iraqi study [28], but disagreed with another [26]. Small sample size of the current study and possible variability in immunohistochemical interpretation could be a cause of these differences in the results. In the current study, it revealed that there was an inverse correlation between ER and Her2/neu, this was in agreement with a study [24] who found that the majority of ER positive and PR positive tumors were Her2/neu negative thus the higher the level of Her2/neu over-expression the lower the corresponding ER and PR positivity. These finding are comparable to another study. [29] Molecular breast cancer subtypes were confirmed by IHC analysis. Luminal B subtype (ER +ve and/or PR +ve, Her2/neu +ve or Her2/neu –ve with Ki67 high score) cases were the most common (42.5%), this agreed with a Moroccan study [30] who recorded (42%) of cases were luminal B subtype, but disagreed with an Iraqi study [31] that recorded (27.8%) of cases were Luminal B. While (22.5%) cases were Luminal A (ER +ve and/or PR +ve, Her2/neu +ve or Her2/neu –ve with Ki67 low score), this agreed with a previous Iraqi study [31] which recorded (24.1%) of cases were Luminal A. Triple negative cases presented (15%) of the studied cases, this finding disagreed with a pervious Iraqi study [31] which found (38%) of cases were triple negative. For Her2 subtype cases were (7.5%) this result was very similar with that of Susan Komen org. [32][33] who estimated Her2 subtype as (10-15%) of all cases of breast carcinoma.

Conclusions:

Ki67 proliferative index represented a valuable tool and provided valuable information about aggressiveness of breast carcinoma and prognosis, significant correlations found between Ki67 and tumor grade, lymph node involvement and Her2/neu score.

Authors contribution:

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