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Immunohistochemistry to Detect Ki-67 ,ER and PR in Iraqi Breast Cancer Patients

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Abstract

Background:Ki67 (cell proliferation marker) has been attracting a considerable attention as a prognostic factor in breast cancer.

***Objective:** To investigate expression and significance of ER, PR and Ki67 in breast cancer, and analyze the influence on judging prognosis.*

***Method :**Immunohistochemistry was employed to detect expression of ER,PR and Ki67 in benign lesions of breast(11 cases) and malignant breast cancer(62cases). All cases were admitted to Al-Yarmouk teaching Hospital, Baghdad Medical City,between December 1999-January2001*

***Results :** Positive rates of ER,PR and Ki67 were 27.2%,45.0%,63.6% respectively in benign lesions of breast. Positive rates of the expression of ER,PR and Ki67 in malignant breast cancer were 37.1%,51.6%and 40.3 % respectively. Poorly Differentiated Ductal Carcinoma(PDC)occupied the highest percentage (50%) for expression of ki-67.*

***Conclusion:** The expression rates of ER, PR and Ki67 in cancer are obviously higher than those in non-*

cancer tissue. In PDC, the expression of Ki67 is higher than any others types of the cancers involved in the study, suggesting that the expression of Ki67 play an important role in malignant transformation of benign lesion of breast. Detection of over expression of all these markers suggests high degree malignancy of the breast cancer.

Key words: *Breast tumors,Ki-67, ER, PR, immunohistochemistry.*

INTRODUCTION:

The leading parameters that define treatment recommendations in early breast cancer are oestrogen-receptor, progesterone-receptor, and human epidermal growth-factor status (1). Ki67, as an easily assessed and reproducible proliferation factor, may be an alternative or complement to histological grade as a prognostic tool and for selection of adjuvant treatment (2). Ki67which is a nuclear protein that is tightly linked to the cell cycle. It is a marker of cell proliferation and has been used to stratify good and poor prognostic categories in invasive breast cancer (3,4). The advent of new genetic tests has emphasised the role of proliferative genes, including Ki67, as prognostic and predictive markers (5).

Despite the absence of standardization of Ki67 pathological assessment, scientists considered the Ki67 labeling index important for selecting the addition of chemotherapy to endocrine therapy in hormone-receptor-positive breast cancers, and classified tumours as low, intermediate, and highly proliferating according to the value of Ki67 (6,7). Assessment of the expression patterns of steroid receptors (ER and PR), and Ki67 in malignant tumors using Paraffin-fixed breast cancer samples using immunohistochemical means was performed by many scientist (8,9). The aim of this study is to compare the expression of prognostically meaningful immunohistochemical markers such as estrogen receptor (ER), progesterone receptor (PR), and Ki-67 in tumor cells of the female patients with breast cancer.

METHODS

Patients:

A total of seventy-three patients presented with breast tumor, were included in this study. The patients were admitted for surgery at , Baghdad Medical City, Al-Yarmok teaching hospital and Al Arabi private hospital. The history as well as personal information, about each patient were obtained through a form which is developed to fulfill the aims of this study .There was informed patient consent, as well as hospital approvals and Ethics committees. Tissues taken from Patients after surgery were fixed in 10%buffered formalin and subjected to routine pathological examination. The patients mentioned were grouped according to their histopathological finding into: Group 1, which included, eleven benign breast tumor patients and Group 2, that included Sixty two malignant breast tumors patients (26 patients Post menopausal, and 36 patients were Pre menopausal).

Ten tissue samples which had no malignant or even benign tumor criteria took during the study were considered as a control. It worth mentioning that tissues samples were taken from the periphery of the site of operation. The histopathological finding of all patients breast tissues were tabulated according to Bloom & Richardson grading system (1957) (10), as presented in Table1.

Table 1: Type of tumor of the specimens under investigation based on bloom & Rechardson (1957)

Group	No. of patients	Type of Tumor
Malignant breast tumors	11	Well differentiated infilterative ductal carcinoma (WDC)
	12	Moderatly differentiated infilterative ductal carcinoma (MDC)
	26	Poorly differentiated infilterative ductal carcinoma (PDC)
	8	In situ ductal carcinoma (IDC)
	5	Infilterative Lobular Carcinom (LC)
Benign breast tumors	4	Fibroadenoma
	6	Fibroadenosis
	1	Epitheliosis

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Sections of 4 mm thickness were cut from representative paraffin blocks containing invasive cancer , and immunohistochemistry was carried out using super sensitive Kit of estrogen receptor (ER) progesterone receptor (PR) and Ki-67, from BIO Genex Company (USA) , and applied as recommended by the manufacturing Company, briefly ,slides were immersed in Retrival citra solution in water bath for 20 minutes , then slides were cooled at room tempreture for 20 minutes after that they were rinsed in PBS (pH 7.2) . The excess of buffer was tapped and wiped around section by clean gauze pad. Endogenous peroxidase activity was blocked by covering the tissue with H_2O_2 in moist chamber for 10 min, followed by 3 washes in PBS. Tapped off excess buffer and whipped around section. Enough power block was added to the section for 10 min in the same chamber. Primary antibodies were added in moist chamber. Then washed with PBS and whipped off around section. Slides were incubated with Biotinlated link antibody for 20 min then washed in PBS and whipped around section. Slides then incubated with labeled streptovidin peroxide for 20 minutes, then washed in PBS and whipped around section. Chromogen/ substrate

were coded as strong expression (++) , weak or moderate expression were coded as (+).

Statistical analysis

ANOVA, T test, Chi sq., were included in statistical processes of all experiments.

RESULTS

As shown in Table 2, the highly percentage of patients with malignant tumors under investigation were recorded in 41-50 years age interval with (38.7 %). Age interval of 51-60 years showed (33.8%), the age interval of 31-40 years, recorded (19.4 %), which reflects the tendency of malignant incidence in younger age. In benign tumor the highest percentage was recorded in age interval of 41-50 years (45.5 %).

was prepared freshly and 4 drops of chromogen plus 2 drops of H_2O_2 and 2-5 drops of working solution were entirely covered the tissue sections and incubated at room temp. for 3-10 min then washed in PBS. The slides were then counter-stained with Harris hematoxylin 1% for (1-2 min). Finally sections were rinsed in gently running tap water for 5 min and dehydrated in Xylene and mounting with D.P.X. then examined under light microscope (40X). Negative control slides were not incubated with primary antibody and the same steps were followed.

Cell counting: Only distinct nuclear staining of invasive carcinoma was used for scoring via light microscope, which determined semiquantitavely as negativel(no immunostining), strong staining

Table 2- Distribution of malignant and benign breast cancer patients according to age

Years of age	Benign		Malignant	
	No.	%	No.	%
20-30	0	0	2	3.2%
31-40	3	27.3%	12	19.4%
41-50	5	45.5%	24	38.7%
51-60	2	18.2%	21	33.8%
61-70	1	9.1%	3	4.8%
Total	11		62	

$P<0.05$

ER was expressed in 37.1% of the malignant tumor cases. High incidence of ER+were detected in PDC which exhibited 46.25% followed by MDC 41.6%. There was no significant differences between benign tumors and malignant tumors ($P<0.5$) for the expression of this marker, but when the malignant tumors values were compared with the healthy control tissue, one could notice a significant differences between them ($P>0.05$) as shown in Table 3.

Table 3: ER status in different types of tumor and in healthy control

Types of tumor	No. of cases	+ER	-ER	Percentage*
IDC	8	2	6	25%
LC	5	1	4	20%
MDC	12	5	7	41.6%
PDC	26	12	14	46.2%
WDC	11	3	8	27.3%
Benign tumor	11	3	8	27.2%
Total malignant	62	23	39	37.1%
Healthy control	10	1	9	10%

% is measured for each as follow $\frac{(+)}{(+)+(-)} \times 100$

P<0.05 Benign x malignant

P>0.05 Control x malignant

Rustles of PR status were mentioned in Table 4, in which, PDC showed the highest percentage of PR (61.5%).

PR were positively expressed in 51.6% of malignant tumors with significant difference when compared to healthy control tissues (P>0.05).

Table 4: PR status in different types of tumor and healthy subjects:

Types of tumor	**No. of cases**	**PR -ve**	**PR +ve**	**Percentage***
IDC	**8**	**4**	**4**	**50%**
LC	**5**	**2**	**3**	**60%**
MDC	**12**	**7**	**5**	**41.6%**
PDC	**26**	**10**	**16**	**61.5%**
WDC	**11**	**7**	**4**	**36.3%**
Benign tumor	**11**	**6**	**5**	**45%**
malignanttumor	**62**	**30**	**32**	**51.6%**
Healthy control	**10**	**9**	**1**	**10%**

P<0.05 Benign x malignant

p>0.05 Control x malignant

shown in Table 5. Malignant tumor expressed ki-67 marker and showed a percentage of 40.3 .There was no significant differences between benign tumor and malignant tumor (P<0.05), whereas As far as ki-67 is concerned with malignant group, PDC occupied the highest percentage (50%), as malignant tumors showed significant difference when compared with healthy control (P>0.05).

Table 5: Distribution of ki-67 values in different types of tumor and healthy control tissue:

Type of tumor	negative for ki-67	Positive for ki-67	Percentage
IDC	7	1	12.5%
LC	4	1	20%
MDC	7	5	41.6%
PDC	13	13	50%
WDC	6	5	45.4%
Benign tumor	4	7	63.6%
Malignant tumor	37	25	40.3%
Control	10	1	10%

P<0.05 Benign x malignant tumor

P>0.05 Control x malignant tumor

Relationship between ER,PR and ki-67

In studying the relationship between ER and ki-67, it was found a significantly positive relationship in PDC (P>0.05) whereas other types didn't reveal any relation.

Concerning PR, there were no significant relationship with this marker (p<0.05), as shown in Table 6.

Table 6: Relationship between ER,PR and ki-67

Type of tumor / **Ki-67**		**ER**		**PR**	
		+	**-**	**+**	**-**
IDC	**-**	**-**	**4**	**1**	**3**
	+	**1**	**-**	**1**	**-**
LC	**-**	**1**	**6**	**3**	**4**
	+	**1**	**-**	**1**	**-**
MDC	**-**	**-**	**4**	**2**	**2**

	+	3	2	3	2
PDC	-	2	5	2	5
	+	11	12	9	4
WDC	-	1	12	7	6
	+	3	2	3	2
Benign tumor	-	-	6	1	5
	+	3	4	4	3
Control	+	-	1	-	1
	-	1	9	2	8

P>0.05 ki-67v ER in PDC

P<0.05 ki-67v ER in other types of tumors.

P<0.05 ki-67v PR in different type of tumors.

Relationship between ER,PR, ki-67 and age

The relationship were demonstrated in Table 7 ,which shows the predominance of ER+ in mean age value 44.7± 9.8 whereas in ER- was 49.6±8.1 , and both showed significant difference in values (P>0.05) ,which reflect the role of the age in the distribution of ER.

In concerning PR, data showed predominance of PR+ in mean age value 49.6±8.1 ,while in PR- were 45.7±9.2 and both showed significant differences (P>0.05), . ki-67 marker also showed significant differences between mean values of patients ages with ki-67+ and ki-67- (P>0.05).

Table 7 : Distribution of ER, PR, ki-67 according to age variation

ER / PR/ki-67	Age	P value
ER+	44.7±9.8	P>0.05
ER-	49.6±8.1	
PR+	45.7±9.2	P>0.05
PR-	50.1±8.2	
Ki-67+	44.4±9.7	P>0.05
Ki-67-	50.2±8.2	

The relationship between different markers to pre and post menopausal status:

In this study all the markers were expressed in relation to pre and post menopausal status of patients. Results were tabulated in Table 8, which showed that all markers (ER,PR and ki-67) exhibited no significant differences in relation to menopausal status ($p<0.05$)

Table 8: Distribution of ER, PR and ki-67 according to menopausal status

Markers	Number of Pre menopausal patients	Number of Post menopausal patients
ER+	17	9
ER-	30	17
PR+	26	11
PR-	21	15
Ki-67+	23	9
Ki-67-	24	17

$P<0.05$ in relation to all markers

DISCUSSION

ER and PR level has been shown to be useful indicator for predicting the clinical response of breast cancer to endocrine ablative procedure. The level of ER and PR in breast cancer tissue has therefore become widely accepted as a guide for the appropriate mode of treatment (11).

In this study 9 of 10 specimens of histologically normal breast tissue were negative for both ER and PR. While 37.1%and 27.2% gave positive results for ER in both malignant and benign tumors respectively, these results were comparable with that gained by (12).Further more 51.6%and 45%of the malignant and benign tumors were positive for PR,and PDC recorded the highest percentage of ER+PR +, different reports showed the diversity in distribution of ER according to type of tumors and this include the present data which showed agree and disagree with many previous reports(2). The expression rates of ER, PR, and Ki67 in cancer are obviously higher than those in non-cancer tissue. Detection of overexpression of all these markers suggests high degree malignancy of the breast cancer these results were in agreement with (13-15).

Nuclear and histological grade have been associated with poor prognosis and this data may

reflect that Ki-67 labeling rate might increase with nuclear and histological grade as indicated in our results and this in agreement with (16). And for this activity, Ki67 could consistently distinguish between different types of breast cancer (17). ER positive/Ki-67 positive tumors were more likely to be high grade (17.7%), this results also recorded by Frédérique Penault-Llorca(18), in which they suggested that such a result may be a hazard for relapse after chemotherapy. The inverse relationship of high Ki67 immunostaining with negative ER immunohistochemistry in the different types of malignant tumors is also consonant with higher grade, highly proliferative tumors being less likely to express ER; and this result coincident with previous study of invasive breast cancers that confirmed a statistically significant inverse association between histological grade and ER status (19). Ki-67 showed positive relationship with ER only in patients with type (PDC). But results gained by (20), were contradictory and showed the lack of any relationship, while (16) ,recorded negative relationship, with poorly-stained tumors with Ki-67 were more likely to be ER,PR positive. The excitement results here is that the high expression of Ki67 molecule in benign tumor, this result could be reflect that this type of tumor may precede to malignant type, because the lower expression of Ki67 in malignant tumors could implicate a less malignant behavior (8).

It is well established that many aspects of immunity change with advancement of age, which may reflect the role of age advancement in the distribution of PR, PR and Ki-67. There are several conflicting reports relating ER content and the age of the patient's breast cancer cases indicated that menopause exerted a greater protective effect on estrogen-receptor negative (ER-) breast cancer than on estrogen-receptor positive (ER+) breast cancer. The continued increase in ER+ cancers after menopause may be explained by both the paracrine growth model and an increase in the proliferation rate of ER+ cells with age. Results found by (20) stated that the age-specific rates of ER- breast cancer cease increasing after 50 years of age, but age-specific rates of ER+ breast cancer continue to increase after 50 years of age.

Postmenopausal breast cancer risk was found to decrease with increasing both hormone receptor positive (ER+/PR) and hormone receptor negative (ER-/PR-) disease, but was stronger for ER-/PR-.

In conclusion, The precise assignment of ER and PR status has an important in the phenotypic analysis of breast tissues and the understanding of the varieties of tumors derived from Ki-67 immunostaining can be considered a convenient method for assessing the prognosis of the disease and we can manage the response of patients to therapy (21, 22).

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