

Immunohistochemical assessment of estrogen, progesterone receptors and HER2/neu overexpression by core needle biopsy in a sample of Iraqi breast cancer patients

Dahlia Raouf Mohammed,¹ Nada A.S. Alwan,² and Raji Hussein¹

¹Medical City, Ministry of Health, Baghdad, Iraq.

²National Cancer Research Center, University of Baghdad, Baghdad, Iraq.

Correspondence to Nada A.S. Alwan (email: nadalwan@yahoo.com).

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Objective The aim of this study was to assess ER, PR and Human Epidermal Growth Factor Receptor 2 (HER2)/neu expression in the specimens obtained by core needle biopsies (CNB) and to correlate the findings with some clinicopathological parameters such as (age, family history, side of breast lump, histological type and grade).

Methods This cross-sectional study was conducted on 62 female patients complaining of palpable breast lumps. All were subjected to the triple assessment test. Patients with mammography or ultrasonography results signifying BI-RADS 4 or 5 were selected for CNB using freehand automated gun instrument. Twenty-three out of 52 malignant cases (44.2%) were with positive scores for ER. Twenty-six (50%) of cases were positive regarding PR expression. HER2/neu overexpression revealed positive scores in 22 cases (42.3%).

Results The correlation between expression of ER, PR and HER2/neu with family history, the side of breast lump and pathological type of carcinoma were statistically not significant. On the other hand, a significant correlation was noted between HER2/neu expression and women's age; the positive expression was more demonstrated among younger aged women (mean age 45 year), nevertheless, this correlation was not significant with ER, PR expression. Significant correlation was also found between HER2/neu overexpression in breast cancer specimens and the histopathological grading, while that association was not maintained for ER and PR expression.

Conclusion Immunohistochemical assessment of hormone receptors and HER2/neu expression in specimens of breast cancer tissues obtained by CNB technique can indicate tumor aggressiveness and further need for neoadjuvant therapy before surgical intervention.

Keywords core needle biopsy, estrogen, progesterone, HER2/neu, immunohistochemistry

Introduction

Breast cancer is the leading cause of cancer death among females in developing countries.¹ In Iraq, it is the most common registered cancer among the society and the main cause of cancer related death among women.² Previous Iraqi studies showed clearly that the disease usually affect middle age females and present relatively at late stages.^{3–6} Reproductive hormones are thought to influence breast cancer risk by increasing cell proliferation, thereby increasing the likelihood of DNA damage, as well as promotion of cancer growth.⁷

Earlier studies suggest that reproductive patterns are more strongly associated with risk of hormone receptor-positive breast cancer compared with triple-negative breast cancer.^{8,9} Human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor (EGFR/ErbB) family. Amplification or over-expression of the *ERBB2* gene occurs in approximately 30% of breast cancers and is associated with increased disease recurrence and a worse prognosis.¹⁰ Signaling through the ErbB family of receptors promotes cell proliferation and opposes apoptosis, and therefore must be tightly regulated to prevent uncontrolled cell growth.¹¹

Core needle biopsy (CNB), also called Tru-cut biopsy, is widely accepted in routine assessment for the diagnosis of breast cancer. It is a reliable method for histological diagnosis and it provides enough material to allow determination for additional markers such ER, PR and HER2 status. Those analyses are critical due to their implications in the guidance of clinical adjuvant proposal.

The aim of this study is to assess ER, PR and HER2/neu expression in specimens obtained by CNB from a sample of Iraqi breast cancer patients and to correlate the findings with some clinicopathological parameters including age, family history, side of breast lump, histological type and grade.

Patients, Materials and Methods

This cross-sectional study was conducted at the Main Referral Centre for Early Detection of Breast Tumors, Medical City, Baghdad and the Iraqi National Cancer Research Centre, Baghdad University. Sixty-two female patients complaining of palpable breast lumps were included in this study. All were subjected to the triple assessment test with physical examination, imaging (mammography and/or ultrasonography) and FNA. Patient with mammography or ultrasonography results signifying BI-RADS 4 or 5 were selected for CNB using freehand automated gun instrument (Bard Mgnun Biopsy Instrument).

At least 2–6 cores were obtained and the specimens were processed and stained with H&E stain for histopathological evaluation. That was followed by further staining with IHC markers ER, PR and HER2/neu. Scoring was based on the examination of all tumor cells on the slide according to Allred scoring guideline for ER and PR. A proportion score (PS) was estimated through assessment of proportion of tumor cells with positive nuclear staining and includes five grades. An intensity score (IS) was estimated by average staining intensity of all positive tumor cells and includes four grades. A total

score (TS) equal to sum of PS and IS. A positive result for both ER and PR is defined as $TS \geq 3$. Weak positivity (score 3–4). Moderate positivity (score 5–6).

Strong positivity (score 7–8). Scoring of the IHC staining for HER2/neu overexpression was according to ASCO/CAP (Hercep test).

Statistical analyses of all results were performed utilizing SPSS software statistical package (version 18) using *t*-test and Chi-square. *P*-value at level of significance <0.05 was used to assess the correlation with some clinicopathological parameters.

Results

This study showed that the peak frequency of breast carcinoma occurred in the age group of 40–49 years. The mean age was 50+ years old. There was an even distribution of breast carcinoma masses in right and left breast (48% and 52%) respectively. About 20% showed positive family history while 80% were with negative family history for breast cancer. Out of total 62 patients, malignant histopathological changes (B5) were displayed in 52 (83.9%) patients, while the remaining 10 (16.1%) patients were non-malignant (i.e., B1, B2, B3 and B4) as diagnosed by CNB technique. Lobular type has been identified in (17.8%) while forty-one cases (78.8%) were IDC in CNB. More than two-third of cases (68%) of IDC by CNB were of grade II.

Twenty-three out of 52 malignant cases (44.2%) were with positive scores for ER, 29 cases (55.8%) were with negative scores. Equal numbers 26 (50%) of cases for positive and negative scores regarding PR expression. HER2/neu overexpression revealed positive scores in 22 cases (42.3%) while negative scores in the remaining 30 cases (57.7%). Regarding the strength of expression for estrogen and progesterone receptors, the highest number of cases with positive scores for ER receptors showed weak positivity while the highest number with PR receptor score revealed strong positivity.

The correlation between expression of ER, PR and HER2/neu with family history, the side of breast lump and pathological type of carcinoma were statistically not significant. A significant correlation was noted between HER2/neu expression and women's age; the positive expression was more among younger aged women (mean age 45 years), on the other hand this correlation was not significant with ER, PR expression (Table 1).

Regarding the correlation with histological type of malignancy; ER PR and HER2/neu receptors expression showed a non-significant statistical correlation with both types (i.e., ductal and lobular carcinoma) for 50 out of 52 malignant cases (as it was not possible to classify two cases by core biopsy diagnosis) as illustrated in Table 2.

While this correlation was not significant with ER and PR expression, as in Table 3.

Discussion

This Iraqi study focuses on immunohistochemical staining for CNB specimens and their correlation with clinicopathological data. Previous studies from the western societies and Iraq showed that the positive expression of ER and PR was increasing with age.^{12–14} In an Iraqi survey, the rates of the positive ER and PR and HER2 breast cancers were 67.8%, 65.3% and 29.4% respectively.¹⁴

Table 1. Distribution of expression of ER, PR, and HER2/neu with respect to age of the patients

Receptor expression	Mean age	SD*	t-Test	Df**	P-value
ER +ve	49.5	11.7	0.51	50	0.61
ER –ve	51.1	11.6			
PR +ve	51.2	11.02	0.44	50	0.66
PR –ve	49.8	12.2			
HER2/neu +ve	45.7	10.1	2.72	50	0.009***
HER2/neu –ve	54	11.4			

*SD = standard deviation. **Df = degree of freedom.

***Highly significant.

Table 2. Distribution of receptors according to the type of mammary carcinoma

Receptor expression	Ductal Ca	Lobular Ca	Odds ratio	Confidence interval	P-value
ER +ve	18	5	0.25	0.05–1.39	0.4
ER –ve	23	4			
PR +ve	19	5	0.76	0.18–3.26	0.71
PR –ve	20	4			
HER2/neu +ve	19	2	3.02	0.56–16.33	0.18
HER2/neu –ve	22	7			

Significant correlation was also found between HER2/neu overexpression in breast cancer specimens and the histopathological grading.

Table 3. Distribution of ER, PR, and HER2/neu according to histopathological grading

Receptor expression	Grade I (n = 3) (%)	Grade II (n = 33) (%)	Grade III (n = 14) (%)	P-value
ER +ve	3 (100)	14 (42.4)	4 (28.6)	0.3
ER –ve	0 (0)	19 (57.6)	10 (64.3)	
PR +ve	3 (100)	16 (48.5)	5 (35.7)	0.28
PR –ve	0 (0)	17 (51.5)	9 (64.3)	
HER2/neu +ve	0 (0)	12 (36.4)	9 (64.3)	0.047*
HER2/neu –ve	3 (100)	21 (63.6)	5 (35.7)	

*Significant.

The difference in the results of the IHC staining among different studies from the same region might be due to technical variations due to the use of different manufacturers and various kits. In the current study, however, no significant association between ER, PR and age was displayed, probably due to the lower number of cases included. On the other hand, a significant correlation was found between HER2/neu overexpression and women's age; the positive expression was more demonstrated among younger aged women (i.e., below 50 year), reflecting the more aggressive behavior of mammary carcinoma in young women.

The correlation of family history with the expression of ER, PR, and HER2/neu was not statistically significant. Studies

suggest an ethnic and racial difference between communities regarding the impact of family history of breast cancer on the expression of hormonal receptors.^{15,16} An Iraqi study carried out to illustrate the clinicopathological features of patients with positive family history of breast cancer revealed that the peak age frequency at the time of diagnosing breast cancer among those patients was in the fifth decade of life; illustrating no distinct correlation for their identification.¹⁷

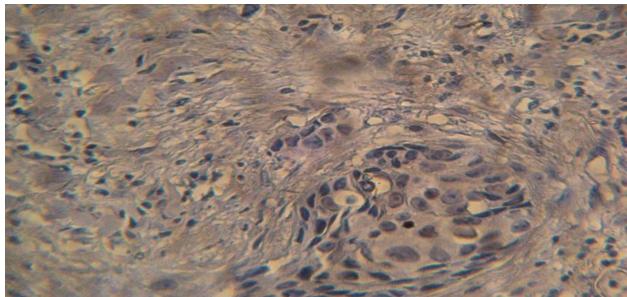


Fig. 1 Immunohistochemical stain for estrogen receptors in invasive ductal carcinoma. Nuclear brown positive staining of tumor cells against a negative cytoplasmic and stromal background. The total score is 5 (moderate positivity) ($40\times + 3.0\times$).

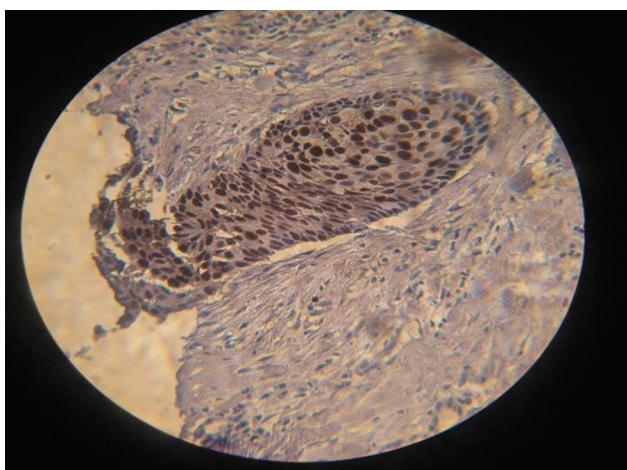


Fig. 2 Immunohistochemical stain for progesterone receptors in invasive ductal carcinoma. Strong nuclear positivity of tumor cells against a negative cytoplasmic and stromal background. Total score is 8 (strong positivity) ($40\times$).

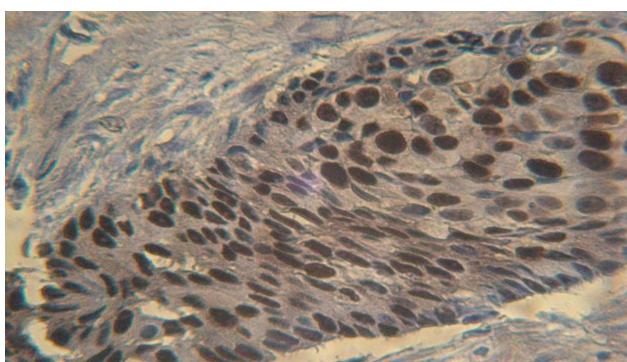


Fig. 3 Immunohistochemical stain for progesterone receptors in invasive ductal carcinoma. Strong nuclear positivity of tumor cells against a negative cytoplasmic and stromal background. Total score is 8 (strong positivity) ($40\times + 3.0\times$).

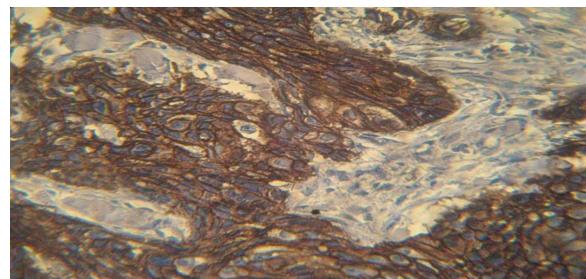


Fig. 4 Strong (3+) membrane immunoreactivity for HER2/neu. A complete membrane staining and strong intensity of more than 30% of tumor cells ($40\times + 3.0\times$).

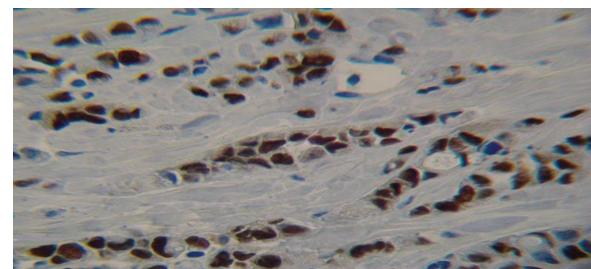


Fig. 5 HER2/neu score 1+ (negative) incomplete membrane immunoreactivity and faint staining in more than 10% of tumor cells ($40\times + 3.0\times$).

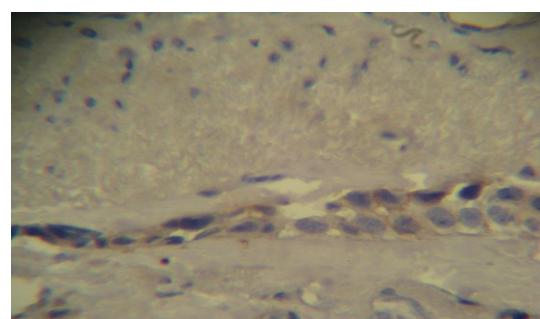


Fig. 6 Infiltrative lobular carcinoma showed strong nuclear positivity for progesterone receptors ($40\times + 3.0\times$).

In this study as well no significant correlation was noted regarding the correlation of ER, PR or HER2/neu with the type of mammary carcinoma. While both progesterone and estrogen receptors did not show any significant correlation with histopathological grading of breast cancer, HER2/neu receptors over expression revealed a significant association. The more the aggressive the tumor was, the more likelihood HER2/neu expression being positive. Similar findings were noted in other local studies.¹²⁻¹⁴

A recent meta-analysis has shown that the CNB tissue could replace open excision biopsy for determining ER, PR, and HER2 status.¹⁸ The 2015 European Society of Medical Oncology breast cancer clinical practice guideline recommends a preoperative pathological examination of the CNB, with a report on ER, PR, and HER2 status by IHC or fluorescence *in situ* hybridization.¹⁹ Similar to previous single center studies,²⁰ the results is subject for selection bias. Some patients were initially diagnosed in other institutes and referred to the surgical department. In those cases, CNB was not reexamined, except in cases of vague diagnoses.

Conclusion

Immunohistochemical staining are readily assessed for hormone receptors and HER2/neu expression in specimens of breast cancer tissues obtained by CNB technique to document tumor aggressiveness and the further need for neoadjuvant therapy. Significant correlations were demonstrated between HER2/neu expression and the age of the patients; higher positive scores were displayed among younger women. Likewise, a statistical association was noted between HER2/neu over expression in breast cancer specimens and the histopathological grading. Nevertheless, no significant correlation was shown between the expressions of ER, PR and HER2/neu and the side of the affected breast, the family history and the histopathological type.

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Recommendations

Further study with larger sample size is recommended to correlate the IHC expression as prognostic and predictive markers in CNB and their significance with clinicopathological parameters and to follow up the patients response to adjuvant therapy.

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