Mouse monoclonal antibodies and immunohistochemical technique for determination of sex steroid receptors and human epidermal growth factor receptor in breast cancer

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Abstract

Breast cancer is one of the most common malignancies in women. The current study was conducted with the objective of assessing estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (Her2/neu) reactivity patterns of breast cancers and to evaluate their association with clinicopathological features. Immunohistochemical (IHC) procedure was used to detect the expression of ER, PR and HER2/neu in postoperative paraffin blocks of breast tumors and statistically analyzed their correlations with clinicopathological characteristics. Most of the patients (66.0%) were ≤50 years at diagnosis. The left breast was more commonly involved (57%), tumor size ranged from 0.5 - 13.0 cm. The staining of Her2/neu was mainly localized in the membrane, whereas ER, PR, were localized in the nucleus. ER positivity was observed in 70% grade I, 48.2% grade II and 3.5% grade III carcinomas ($P<0.05$). Similarly PR positivity was observed in 70% grade I, 36.14% grade II and 1.75% grade III carcinomas ($P<0.05$). HER2/neu was positive in 1 (10%) case of grade I carcinoma, 31 (37.35%) cases of the grade II carcinoma and 24 (42.11%) cases of grade III carcinoma. In the HER-2/neu positive tumors, ER and PR expression in high grade tumors was significantly decreased compared with intermediate grade tumors (ER 5.6% vs 10.5; PR 0% vs 5.3%). Among the ER, PR and HER2/neu statuses, a significant correlation was observed between ER expression and PR status ($P<0.05$), whereas the expression of ER and PR exhibited a negative correlation with HER2 status ($P<0.05$). We also demonstrated a significant correlation between the HER2/neu subtypes with poor histological grade ($P<0.05$). In conclusion, there is a definite correlation between IHC indices and clinicopathological characteristics in breast carcinomas.

Keywords: Brest cancer; Progesterone; Estrogen; Her2/neu

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Introduction

Breast cancer is the most common cancer among women, accounting for one in four types of cancer diagnosed in women worldwide. It is the main cause of death in women 45 to 55 years of age. Over 1.1 million women are diagnosed with this disease each year and incidence rates are still on the increase in several countries [1, 2]. The ultimate outcome of breast cancer relies on its initial stage at diagnosis with the main prognostic factors associated with breast cancer being lymph node involvement, tumor size and histological grade [3, 4]. Therefore; it is important to find biomarkers that will predict the likelihood of recurrence and identify those patients who might benefit from additional therapy. Hence, low-risk patients can be spared unnecessary and costly treatment. Moreover, high-risk patients could be rapidly identified and offered appropriately aggressive treatment [5, 6].

Estrogen and progesterone receptors (ER, PR) and more recently, HER- 2/neu have with increasing importance influenced the management of the malignancy [7-11]. ER and PR have a crucial role in the proliferation and progression of breast cancer [4]. Estrogens are potent mitogens that mediate its proliferative action through the induction of cyclin D1, the major regulator of entry into the G1 stage of the cell cycle, and promote the secretion of positive or negative paracrine growth signals by breast stroma cells, stimulating epithelial cells to proliferate [4]. With an established positive correlation of ER and PR with the degree of tumor differentiation, determination of ER and PR status on biopsy specimens prior to therapeutic intervention is advocated as standard practice [8]. HER-2/neu also known as C-erb B2 (HER-2), is a proto-oncogene located on chromosome 17 [9, 10]. It is amplified and the protein (HER-2) overexpressed in 15-25% of invasive breast carcinoma with associated poor prognosis. HER-2/neu encodes a transmembrane glycoprotein with tyrosine kinase activity known as p185 belonging to the family of epidermal growth factor receptors [9, 10].

Immunohistochemical (IHC) detection has become essential to many malignancies and plays a key role in tumor diagnosis, treatment and prognostic assessment [11]. In this study, we study 75 cases of invasive BC and 15 of benign proliferative diseases, to detect the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2), by IHC and analyzed the associations between these indicators and the clinico- pathological characteristics.

Materials and methods

Breast samples used in this study were obtained by total or partial mastectomy in Hilla Teaching Hospitals, Babel, IRAQ during a ten years period extending from 1st January 2005 to 1st January 2014. They included a total of 75 cases of infiltrative carcinoma and 15 of benign proliferative diseases including ductal and lobular hyperplasia, apocrine metaplasia, fibroadenoma, and fibrocystic changes. Four micrometer thick formalin fixed, paraffin embedded tumor sections were stained with Haematoxylin and Eosin. Histological grade was assessed according to Nottingham modification of the Bloom-Richardson system. The presence of invasive carcinoma was confirmed in all cases. The histologic type of each tumor was

recorded. All infiltrative tumor samples were classified by the TNM system [12]. Each specimen was processed for immunohistochemistry using formalin fixed and paraffin embedded tissues sections and the hormonal status of each lesion was evaluated. Representative sections with breast tissue tumor were processed for ER, PR and HER-2/neu immune-histochemical staining [13]. Sections 4 μm thick were processed by using the avidin–biotin–peroxidase complex method (Fig.1). After the removal of paraffin, sections were hydrated and incubated for 30 min in 0.3% H2O2 to inhibit endogenous peroxidase activity; to retrieve the antigen the sections were incubated with retrieval solution (Dako Cytomation, Carpinteria) and heat at 90°C in a vegetable steamer for 10 minutes.

After being rinsed in Tris-buffered saline (TBS), the slides were incubated with 3% normal rabbit serum (NRS) in TBS for 30 min to prevent non-specific binding of the first antibody. The sections were then incubated with primary mouse monoclonal antibodies, ER (dilution 1:25) (Dako Cytomation) and PR (DakoCytomation) (dilution 1:100), for 30 minutes at room temperature. For HER-2/ neu staining, after antigen retrieval, slides were stained with a polyclonal antibody against HER-2/neu (DAKO) oncoprotein by envision system. HER2 score of 3+ was taken as positive. A score 3+ may be taken as "positive" as over 90% of these show gene amplification.

Statistical analysis

The results of principal components analysis were confirmed by determination of the correlation of ER, PR and HER- 2/neu receptors expression status and several clinic-pathological factors, by using Chi-square tests ($\chi 2$ test two-tailed). Fisher's exact test were also performed to examine whether or not the studied variables differed. A P value of <0.05 was taken as significant.

Results

A total of 75 breast cancer cases were included in the study. The mean age was 47.3 years (range 31-76 years; median age 44.5 years). Most of the patients (66.0%) were ≤50 years at diagnosis. The left breast was more commonly involved (57%), tumor size ranged from 0.5 - 13.0 cm and immuno-histochemical stained slides were evaluated for the presence of positive reaction, cellular localization (nuclear or cytoplasmic), pattern of staining (focal or diffuse), and intensity of reaction in individual tumor cells (strong or weak). The intensity of positive nuclear reactions was evaluated against the reaction in respective control samples. The staining of HER2 was mainly localized in the membrane (Fig. 2), whereas ER, PR, were localized in the nucleus (Fig. 3, 4 respectively).

Cells were classified according to the positive rate and color intensity as follows: negative (-), weak positive (+), moderate positive (++) and strong positive (+++). The morphological categories were infiltrating ductal carcinoma (IDC) 64 (85.3%) cases, metaplastic carcinoma 3 (4.0%) cases, IDC with mucinous differentiation 2 (2.7%) cases, infiltrating lobular carcinoma 1 (2.0%) case; mixed (ductal and lobular) carcinomas, IDC micropapillary and medullary carcinoma 1 (1.3%) cases each. Other types were apocrine, papillary and pleomorphic lobular carcinoma accounting for a single case each. Tables 2 and 3 give the ER, PR and HER-2/neu status by tumor grade, size and lymph node involvement. Seven (9.3%) cases were grade I, 40

(53.3%) were grade II and 28 (37.3%) were grade III. ER and PR were positive in 49 (32.7%) and 38 (25.3%) cases respectively. HER-2/neu was positive (3+) in 37 (24.7%), 2+ in 19 (12.7%) and negative (0 and 1) in 94 (62.7%) cases. Simultaneous ER and HER-2/neu positivity was observed in 6 (4%) cases. Special sub-type of carcinomas like metaplastic carcinoma showed no HER-2 positivity, except for a case of grade II IDC with mucinous differentiation. ER and PR expression correlated inversely with HER-2 over-expression.

ER and PR expression were decreased significantly in HER-2/neu positive compared with HER- 2/neu negative tumors (table 1, 2). ER positivity was observed in 71.4% grade I, 50 % grade II and 3.5% grade III carcinomas (P value <0.005). Similarly PR positivity was observed in 70% grade I, 36.14% grade II and 1.75% grade III carcinomas (P value<0.005). HER-2 was negative in all cases of grade I carcinoma, 9(22.5) cases of the grade II carcinoma and 10(35.7) cases of grade III carcinoma. In the HER-2/neu positive tumors, ER and PR expression was significantly decreased (table1). ER positivity was observed in 71.4% grade I, 50% grade II and 3.5% grade III carcinomas (P value <0.001). Similarly PR positivity was observed in 71.4 grade I, 37.5% grade II and 3.50% grade III carcinomas (P value<0.001). HER-2 was positive in None case of grade I carcinoma, 31 (22.5%) cases of the grade II carcinoma and 35.7% cases of grade III carcinoma. In the HER-2/neu positive tumors, ER and PR expression in high grade tumors was significantly decreased compared with intermediate grade tumors. With regard to tumor size, three groups was performed, group 1 (tumor size < 2 cm), group 2 (2–5 cm) and group 3 > 5 cm in diameter. The total number of group 1 tumors was 9 [5 (55.5%) ER positive; 4 (44.4%) PR and 2(22.2%) Her-2 positive. Of the group 2 tumors 14 (35%) were ER positive, 12 (30%) were PR positive and 9 (22.5%) were HER-2 positive. Of the group 3 tumors 7 (20%) were ER positive, 5 (14.2%) were PR positive and 8 (23%) were HER-2 positive. ER expression in the HER-2/neu positive, large sized tumors was significantly decreased compared with smaller tumors (ER 20% vs. 55.5).

	ER+ (#26)	PR+ (#21)	HER/2neu+ (#19)
Grade 1 (#7)	5(71.4)	5(71.4)	None
Grade 2 (#40)	20 (50)	15(37.5)	9(22.5)
Grade 3 (#28)	1 (3.5)	1(1.75)	10(35.7)
p-value<0.005			
<2.0 cm (#9)	5(55.5%)	4(44.4)	2(22.2)
2-5 cm (#40)	14(35%)	12(30)	9(22.5)
>5.0 cm (#35)	7(20%)	5 (14.2)	8(23)
p-value<0.005			
No LN (#22)	6 (27.2%)	5(22.6)	3(13.6)
1-3 LN (#17)	7 (41.1%)	5(29.4)	5(29.4)
> 3 LN (#36)	13(36.1%)	11(30.5)	11(30.5)
p-value <0.005			

(Positive: +)

Table 1.
Correlation of ER, PR and HER-2/neu Status with malignant Grade, tumor Size and Lymph Node metastasis.

	ER expression				PR expression			
HER-2neu status	+(%)	++(%)	+++(%)	- (%)	+(%)	++(%)	+++(%)	-(%)
HER-2/neu positive (3+;19)	6	4	10	80	6	10	4	80
HER-2/neu positive	6	23	23	48	14	14	14	58
HER-2/neu negative (47)	5	5	24	66	5	5	18	72

Table 2.
Correlation of HER-2/neu Status with Estrogen and Progesterone Receptor Expression.

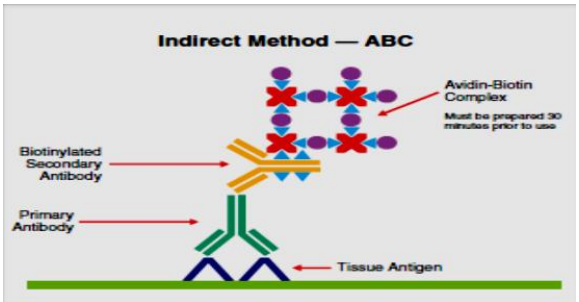

Figure 1.
Immuno-histochemical (IHC) procedure-Avidin-Biotin Complex (ABC) Method.

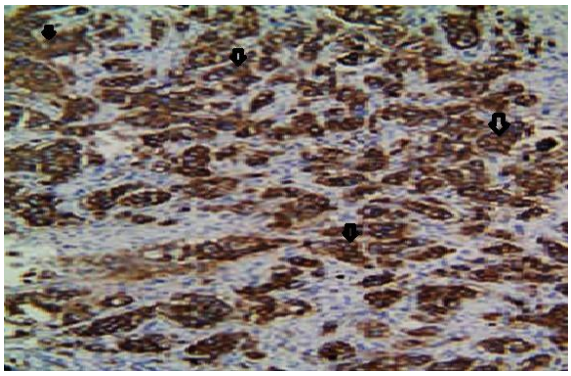

Figure 2.
Positive immunohistochemical expressions of HER-/neu in invasive breast cancer tissues, existing mainly in the membrane (arrowed) (Magnification, ×200).

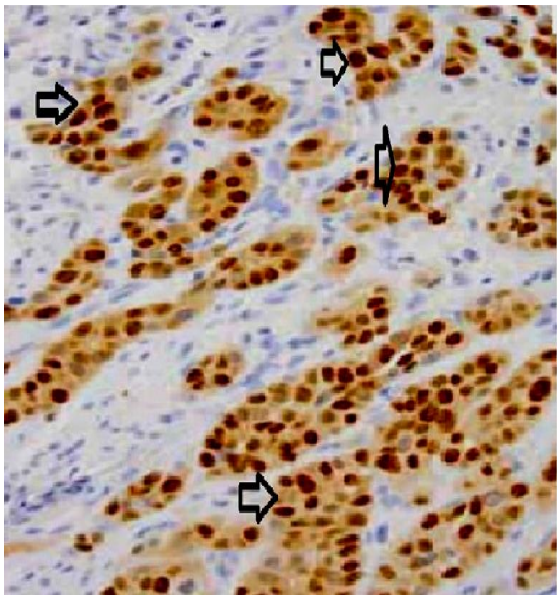

Figure 3.
Positive estrogen receptor (ER) expressions in invasive breast cancer tissues. ER are detected only in the nucleus. (Magnification, ×400).

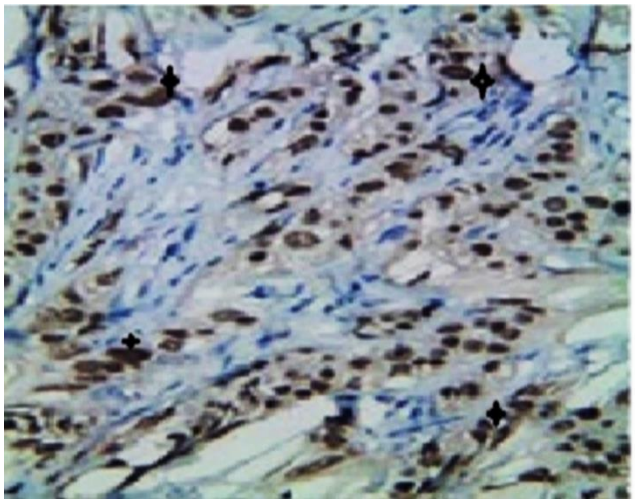

Figure 4.
Positive immunohistochemical for progesterone receptor (PR) expressions in invasive breast cancer tissues. Progesterone receptor (PR) are detected only in the nucleus (stars), (Magnification, ×400).

Discussion

Breast cancer survival is linked to early detection, timely appropriate treatment and genetic predisposition. Prognosis is related to a variety of clinical, pathologic and molecular features which include classical prognostic factors vs. histologic type, grade, and tumor size and lymph node metastases [14-19]. In the present study data regarding tumor size, type, histological grade, lymph node status and hormone receptor status as well as HER2 marker status for breast carcinomas were analyzed.

The age at the time of diagnosis of breast tumors was 47.3 years .The mean age at diagnosis for malignant breast tumors in our study was similar to that of another study. A third of the cases presented with large, necrotic tumors. The morphology was infiltrating ductal carcinoma with grade II or grade III disease, and lymph node involvement. These demographic findings complement other studies which have

stressed on the younger age of breast cancer cases at presentation and the higher stage and tumor grade [14-19]. With regard to hormone receptors, ER were positive in 71% for grade I and 50% for grade 2 breast cancer cases respectively and PR were positive in 71% for grade I and 37.5% for grade 2 cases respectively. Regarding HER2 expression, HER-2/neu was nil in grade I, positive in 22.5% for grade II and positive in 35.7% for grade III breast cancer cases respectively.

We compared our results with previously published data [19-23]. A studies of invasive breast cancers conducted by other groups has reported a similar HER-2/neu over-expression results [24-28]. In our study, expression of ER and PR were decreased significantly in HER-2 positive tumors in comparison with HER-2 negative tumors, however, a substantial number of HER-2 positive tumors still expressed ER or PR, an observation similar to the other reported study [29-31]. In the study HER-2 positivity was limited to invasive breast carcinomas of the ductal and lobular morphology, in the pleomorphic sub-types, not in the classic variety [24]. None of the special type carcinomas like mucinous, metaplastic and adenoid cystic types showed HER-2 positivity [24]. These findings are similar to our study. In both studies HER-2 positivity was associated more strongly with higher histologic grade carcinoma. None of the grade I carcinomas were HER-2 positive in both studies; the majority of grade III tumors expressed positivity whereas a smaller component of grade II carcinomas were HER-2 positive [25-29].

Correlation of HER-2/neu over-expression and tumor grade was also studied with a sample cases of invasive breast carcinomas. According to their study, HER-2/neu over-expression was associated with a higher tumor grade [25]. Similarly of other study show that the over- expression of HER-2/neu in 29.7% of breast cancers, significantly correlating with larger tumor size and a decreasing level of ER [26], while another study provided comparable results [27]. The ER, PR expression in breast cancer, in the current study is comparable to published international studies, but the frequency of HER-2neu expression is higher in the current study. This may reflect the younger age at diagnosis. Larger studies are required to study the biological behavior of breast cancer in this high risk population. The clinical importance of these prognostic markers in the management of breast cancer patients is strongly advocated in our population to improve the dismal prognosis and to provide better therapeutic options.

In conclusion, there is a definite correlation between IHC indices and clinico-pathological characteristics in breast carcinomas. Combined detection of these indices may be significant in the evaluation of biological behavior and prognosis of breast carcinomas and thus in the diagnosis and comprehensive treatment of this disease.

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