American Journal of BioMedicine
2016; 4(3): 168- 179

Immunohistochemical determination of estrogen and progesterone receptors in breast cancer: pathological correlation and prognostic indicators

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Abstract

Carcinoma of the breast is the most common malignancy of women globally and the incidence has more risen in recent years. The current study was conducted with the objective of assessing estrogen receptor (ER) and progesterone receptor (PR) reactivity patterns of mammary cancers and to evaluate their association with clinicopathological features. A total of 61 cases of breast carcinoma were examined retrospectively using immunostains for estrogen receptor (ER) and progesterone receptor (PR). Staining pattern and intensity were correlated with histological subtypes and nuclear grades of tumors. The left breast was more commonly involved (57%) and tumor size ranged from 0.5-13.0cm. The predominant morphology was infiltrating ductal carcinoma (85.3%). The majority of the cases presented as grade II (55.3%) lesions with tumor necrosis (70%) and lymph node involvement (71.3%(. Positive nuclear staining for ER and PR was observed in 70.5% and 57.5 % of invasive carcinomas, respectively. In ER+ cases, fifty five cases (90%) gave diffuse immunohistochemical reaction for ER; in the remaining 10%, a focal ER reaction was seen. In PR+ cases, 49 cases (80%) gave diffuse immunohistochemical reaction for PR and In remaining 20% of PR+ tumors, the reaction was heterogeneous. In ductal infiltrative carcinomas the percentage of cases showed ER+ nuclear labeling is higher than those in cases of infiltrative lobular carcinomas. Assessment of ER and PR as prognostic markers for the clinical management of breast cancer patients is strongly advocated to provide best therapeutic options.

Keywords: Breast cancer; Estrogen receptor (ER); Progesterone receptor (PR)

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Received January 30, 2016; Accepted June 25, 2016; Published August 08, 2016

Immunohistochemical reaction; Prognostic markers

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Introduction

Breast cancer is the most common cancer among women globally, and is the main cause of death in women 45 to 55 years of age [1]. It accounts for a third of the cancer in female with an age standardized incidence rate of (ASR) world of 53.8 per 100,000 population annually [2]. Steroids are necessary for normal breast development. An imbalance precipitates abnormal pathological processes including epithelial hyperplasia, dysplasia, intraductal and invasive carcinoma [3, 4].

Estrogen is an important mitogen exerting its activity by binding to its receptor (ER) and found in 50-80% of breast cancer. Analysis of steroid receptor status has become the standard of care for patients with breast cancer. Estrogen receptor (ER) content, in particular, has been correlated with prolonged disease-free survival and increased likelihood of response to endocrine therapy. Assessment of ER status by immunohistochemical analysis has been shown to have higher discriminating power for predicting overall and disease-free survival [5-9].

Endocrine treatments are assigned to antagonize the effects of estrogen. Therapeutic hormones competitively block ER thus antagonizing transcriptional activation of genes required for tumor growth [10, 11]. The presence of hormone receptors (ER and PR) in the tumor tissue correlates well with the response. Studies have shown that 55-60% of women with ER-positive tumors respond to additive or ablative hormone therapy, compared with about 8% of women with ER-negative tumors. Tumors that are better differentiated are more likely to be ER and PR positive and have a relatively better prognosis [12, 13].

The objectives of this study were to assess the ER and PR reactivity pattern in breast carcinomas based on the immunohistochemical staining of 61 breast cancer cases for ER and PR during a 10-year period and to correlate this reactivity pattern with the various pathological factors to improve our knowledge of the influence of these receptors on the development and progression of breast cancer and their possible influence in endocrine therapies.

Materials and methods

Tissue sample

Breast samples used in this study were obtained by total or partial mastectomy in Hilla Teaching Hospitals, Babel, Iraq during a six years period extending from 1st January 2006 to 1st January 2012. They included a total of 61 cases of infiltrative carcinoma and 15 of benign proliferative diseases including ductal and lobular hyperplasia, apocrine metaplasia, fibroadenoma, and fibrocystic changes. *Hematoxylin and Eosin (H&E)*

H&E-stained slides of each case were reviewed, and the presence of invasive carcinoma was confirmed in all cases. The histological type of each tumor was recorded. All infiltrative tumor samples were classified by the TNM system [14].

Immunohistochemistry

Each specimen was processed for immunohistochemistry using formalin fixed and paraffin embedded tissues sections and the hormonal status of each lesion was evaluated. Sections 4μm thick were processed by using the avidin–biotin–peroxidase complex (ABC) method. After the removal of paraffin, sections were hydrated and incubated for 30 min in 0.3% H_2O_2 to inhibit endogenous peroxidase activity; to retrieve the antigen the sections were incubated with retrieval solution (DakoCytomation, Carpinteria, CA) and heat at 90°C in a vegetable steamer for 10 minutes [15, 16]. 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 nonspecific binding of the first antibody. The sections were then incubated with primary mouse monoclonal antibodies, ER (dilution 1:25) (DakoCytomation) and PR (DakoCytomation) (dilution 1:100), for 30 minutes at room temperature. The sections were washed in TBS and incubated for 30 minutes with the linking solution; biotinylated antimouse immunoglobulin; the sections were incubated with avidin– biotin–peroxidase complex (Dako) for 30 minutes and developed with 3,3'diaminobenzidine (DAB), using the glucose oxidase–DAB–nickel intensification method [17]. Sections were dehydrated and mounted in DePex.

Specificity of the immunoreactions

To assess the specificity of the immunoreaction, negative and positive controls were used, tumor blocks were selected that contained normal or non-neoplastic mammary epithelium to serve as positive internal control samples. The external positive control

samples for ER and PR were cases of invasive mammary carcinomas. The antibody (negative) control sample consisted of replacement of the primary antibody with nonimmune mouse IgG on adjacent histological sections, or using the antibody pre-absorbed with an excess of purified antigens, or omitting the primary antibody.

Statistical analysis

The data were entered and analyzed in SPSS version 16. Frequencies and percentages of categorical variables; mean and standard deviation of quantitative variables of the different pathologies studied were computed. A p value of <0.05 was taken as significant, as calculated by applying correlation coefficients and multiple logistic regression. The results of principal components analysis were also confirmed by determination of the correlation of ER and PR receptors expression status and several clinical and pathological factors, by using Fisher's exact test or the $\chi 2$ test (two-tailed). The final treatment strategy included life-long folic acid supplementation, periodic blood transfusion support and/or splenectomy, as indicated.

Results

Receptor	**No.**	**(%)**
ER+	41	(67)
PR+	30	(49)
ER+/PR+	30	(49)
ER+/PR–	10	(16)
ER–/PR–	13	(21) ER–/PR+
0	(0)	

ER, estrogen receptor; PR, progesterone receptor; +, positive; –, negative.

Table 1.
ER and PR status in 61 cases of breast carcinoma.

Subtypes of carcinoma	ER+ No (%)	PR+ No (%)
Infiltrating ductal (n = 45)	32 (71)	26 (57)
Infiltrating lobular (n = 5)	5 (100)	3 (77)
Tubular (n = 3)	3 (100)	3 (100)
Colloid (n = 2)	2 (100)	2 (100)
Papillary (n = 1)	1 (100)	1 (100)
Apocrine (n = 1)	0 (0)	0 (0)
Medullary (n =2)	0 (0)	0 (0)
Metaplastic (n = 2)	0 (0)	0 (0)

ER, estrogen receptor; PR, progesterone receptor; +, positive.

Table 2.
Correlation of estrogen and progesterone receptor status to histological subtypes of breast carcinoma.

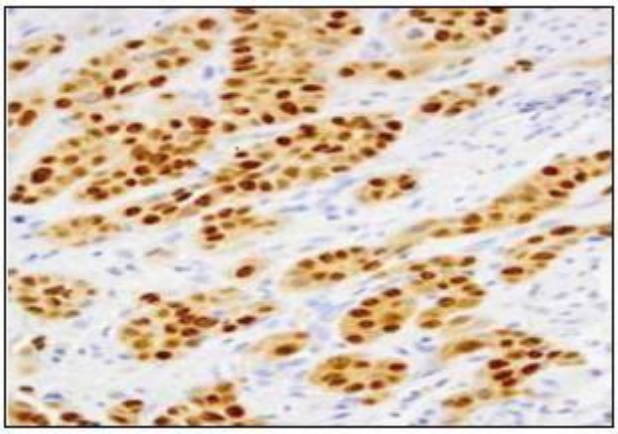

Figure 1.
Infiltrating ductal carcinoma, intermediate nuclear grade. Uniform positive reaction for Progesterone Receptors in tumor nuclei (×200).

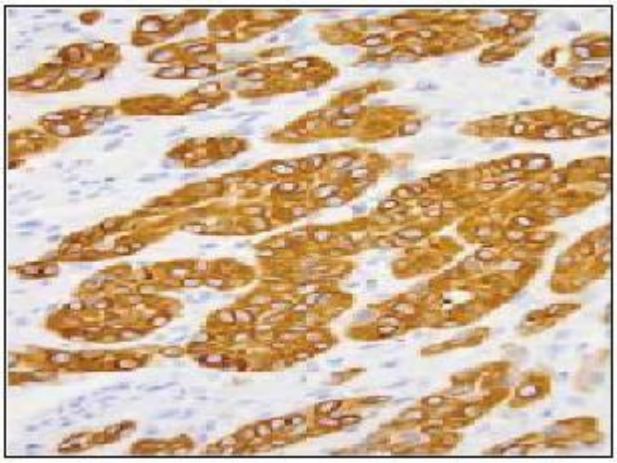

Figure 2.
Infiltrating ductal carcinoma, high degree nuclear grade. A strong nuclear reactivity to ER in tumor nuclei (×400).

Grades	**ER positive (#43)**	**PR positive (#35)**
Grade 1 (#10)	8 (80.0)	8 (80.0)
Grade 2 (#32)	25 (78.2)	20 (62.5)
Grade 3 (#19)	10 (52.6)	7 (36.8)

Table 3.
ER and PR Status by Tumor Grade .

Clinical and pathological

Patients A total of 61 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.

Immunohistochemical reaction

Immunohistochemically 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). Any positive nuclear reaction for ER and PR, irrespective of percentage of reactive cells, was recorded as positive. The intensity of positive nuclear reactions was evaluated against the reaction in respective control samples.

The frequency of steroid receptor status of ER and PR in 61 cases of infiltrating breast carcinoma and the correlation of estrogen and progesterone receptor status to histologic subtypes of breast carcinoma is summarized in tables 1,2. Fifty five cases (90%) gave diffuse immunohistochemical reaction for ER and 49 cases (80%) gave diffuse immunohistochemical reaction for PR. In benign breast diseases no immunoreaction for ER or PR was observed. The immunohistochemical study showed no reaction in the negative controls incubated with the pre-immune serum, using the antibody pre-absorbed with an excess of purified antigen, or omitting the primary antibody.

ER reaction

A positive reaction for ER was observed as brown-black, fine, intranuclear granules. The staining reaction in the breast cancer cases (invasive ductal and lobular) for PR was heterogeneous, strongly positive and weakly positive, and sometime negative nuclei were seen side by side or grouped together (Figure 1). In contrast, the staining reaction for ER in most invasive mammary carcinomas that stained positively for this receptor was diffuse and uniform. In positive cases, the intranuclear staining reaction for ER was observed in more than 90% of the tumor cells throughout the lesion (Figure 2). The overall intensity of the reaction in a given tumor usually was high. Variation in the intensity of the positive reaction among tumor cells was minimal. This phenomenon was best illustrated in well-fixed core biopsy specimens.

Correlation of estrogen and progesterone receptor status to histological grade of breast carcinoma is summarized in (Table 3). ER positivity was observed in 80% grade I, 78.2% grade II and 52.6% grade III carcinomas (*P* value<0.005). Similarly PR positivity was observed in 80% grade I, 62.5% grade II and 36.8% grade III carcinomas (*P* value<0.005).

Discussion

Breast cancer is the most common cancer among women , and is the main cause of death in women 45 to 55 years of age worldwide [18, 19]. 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]. However, tumor at the same stage can behave in a different

manner, and the prognosis can vary [20-23]. 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.

Sex Steroid Receptor (SSR) have a crucial role in the proliferation and progression of breast cancer [6]. Estrogens are potent mitogens that mediate its proliferative action through the induction of cyclin D_1, 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 [7-9].

In this study we observed that a high proportion of infiltrative ductal carcinomas and infiltrative lobular carcinoma cases showed nuclear immunostaining for ER and or PR; fifty five cases (90%) gave diffuse immunohistochemical reaction for ER and 49 cases (80%) gave diffuse immunohistochemical reaction for PR. This finding contrasts with the absence of immunoreaction in benign proliferative diseases samples. These results are in agreement with those observed by Palmieri and colleagues [20] who found low ER-α expression in fibrocystic disease and high ER-α expression in invasive ductal cancer.

Most breast cancers showed relatively homogeneous staining for ER, whereas the expression of PR was heterogeneous and focal in more than 20% of the cases. Variations in the staining pattern for ER occurred but in most cases could be attributed to factors related to tissue fixation and antigen preservation. In general, with prolonged fixation (more than a few days), there is an exponential decrease in the sensitivity of the immunohistochemical stains [24]. Heat-induced antigen retrieval can help reduce this problem, but it is ineffective in restoring the antigen that is lost owing to inadequate fixation, i.e., lack of complete penetration of fixative into the center of the specimen. False-negative results might occur in unfixed areas, particularly when there is fibrosclerotic tumor stroma. This observation is supported by a recent report by Goldstein and colleagues [25].

Most breast carcinomas are diffusely positive or completely negative for ER-$1D_5$. One may argue that this observation could be the result of an inordinately high sensitivity of the immunohistochemical system in this study. Uniform expression of ER

and most other biomarkers in breast cancer reflects current concepts about the biology of these neoplasms. human mammary carcinomas are monoclonal in origin [26, 27]. This is in contrast with other common cancers such as prostatic carcinomas in which the great majority are polyclonal and phenotypically heterogeneous [28]. The quantitative variability seen with PR, on the other hand, might be a reflection of functional variability of ER in some breast cancers.

The expression of ER & PR expression in breast cancer cells is crucial in determining whether antiestrogen therapy would be efficient, because some antiestrogenic drugs such as 4-OH-tamoxifen are more competitive than other estrogenic substance antagonists of ER-β [29], and do not display agonist activity when the receptor concentration increases. ER-β therefore seems to suppress the partial agonist activity of tamoxifen on ER-α [30], so the response to this treatment will evidently depend on the distribution of these receptors. In our study, we found that those patients showed negative ER & PR expression were usually not the same as those who showed a positive ER & PR expression, and most negative ER & PR expression patients had a poor prognosis and a worse response to hormonal therapy than a positive ER & PR expression patients.

In a similar study, Fatima *et al* [4] showed 55% ER and PR reactivity, while in our study ER reactivity was seen in only 32.7% of invasive breast cancers. In their study, ER positivity decreased with increasing tumor size and grade, however, no significant correlation was seen with lymph node metastasis. Similarly we found that ER positivity decreased with increasing tumor size and grade. The ER, PR expression in breast cancer, in the current study is comparable to published international studies, but the frequency of 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.

In conclusion, 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.

Competing interests

The authors declare that there is no conflict of interest.

Author Contributions

All authors wrote, read and approved the final manuscript.

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