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The Value Of Immunohistochemical Expression Of Estrogen Receptor And Progesterone Receptor Receptors In The Prognosis Of Iraqi Women With Breast Carcinoma

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ABSTRACT

Background: The basic microscopical structure of the adult female breast is composed primarily of two components. The first is an active adipocyte (mesenchyme). The second is an epithelial bilayer, which is supported by Cooper ligaments (a loose framework of dense fibrous connective tissue). Immunohistochemical evaluation of breast tissue is of diagnostic, prognostic, and therapeutic relevance. Estrogen receptor (ER) and progesterone receptor (PR) are playing a vital role in the management of breast cancer because they predict response to endocrine treatment; however, they have a little prognostic role regarding the fate of disease. These receptors had assessed by immunohistochemistry (IHC) that had performed on paraffin tissue preparations.
Aim of the study: The current study was aiming at highlighting the value of immunohistochemical expression of ER and PR receptors in the prognosis of Iraqi women with breast carcinoma.
Materials and methods: The current study was carried out at the central laboratory of Al-Diwaniyah teaching hospital and several private histopathology laboratories in Al-Diwaniyah province, the mid-Euphrates region of Iraq. The study was started in July 2019 and ended in February 2020. Thirty-paraffin sections of breast carcinoma had retrieved from these laboratories. One-section had been made from each paraffin block and stained by the conventional hematoxylin and eosin (H and E) stain. Then, the sections had reviewed by two pathologists, who confirmed the diagnosis, as well as the other related histological features like grade, histological subtype, stromal invasion, and lymphatic and neural permeation. Two-further sections had made for immunohistochemical staining with ER and PR receptor stains.
Results: The immunohistochemical expression of ER and PR receptors was as following: Positive ER only cases were 3 (10.0 %), positive PR only cases were 4 (13.3 %), and the cases which were positive for both ER and PR were 15 (50.0 %). The Cases that were negative for both ER and PR accounted for 8 (26.7 %). Total cases expressing ER accounted for 18 (60.0 %), and the cases that express PR had accounted for 19 (63.3 %).
Conclusion: The immunohistochemical expression of ER and PR receptors is heterogeneous concerning the proportion and intensity of staining, and this may explain the variation of breast cancer cases in response to hormonal therapy.

Keywords: immunohistochemical expression ER, PR, prognosis, breast, carcinoma

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INTRODUCTION

The mammary glands are "modified tubuloalveolar apocrine sweat glands located on the anterior thoracic wall" [1-3]. Throughout the classical female age, the structure of these glands changes considerably (specifically) during puberty, pregnancy, menstrual cycle, and menopause [4, 5]. Until the onset of puberty, male and female breasts are the same, but at puberty, the mammary epithelium passes down to the breast fat pad and starts proliferation; however, this process is inhibited in the male by the effect of rising testosterone hormone [1-5]. The basic microscopical structure of the adult female breast is composed primarily of two components. The first is an active adipocyte (mesenchyme). The second is an epithelial bilayer, which is supported by Cooper ligaments (a loose framework of dense fibrous connective tissue) [3-5]. The epithelium forming the ducts is composed of inner epithelial cells and outer myoepithelial cells. These ducts then radiate to lobes (15-20) and lobules (20-40) and terminate at the terminal ductal lobular units (TDLUs) [1-6].

In women, breast tissue is the site of a common malignant disorder called cancer of the breast. Breast tissue can be affected by benign tumors that remain localized and have an excellent prognosis, such as fibroadenoma [7-9]; however, malignant tumors that invade surrounding tissues and spread through blood and lymphatics also affect breast tissue [9-11]. Breast cancer has regarded as the most common cancer affecting women and the most common cause of cancer-related death in women [10, 12]. Several risk factors are associated with the development of breast cancer. Regarding age, the disease is rare before the age of 25 years. The incidence usually increased with increasing age, although the median age at the time of diagnosis is during the 60s, and most cases have diagnosed after the age of 50 [13]. Previous breast proliferative lesions also increase the risk of breast cancer [14]. A family history of breast cancer also increases the risk of breast cancer [15]. Early menarche, late menopause, nulliparity, and lack of breastfeeding has regarded as risk factors for breast cancer [10]. Exogenous hormone replacement therapy has associated with a higher

risk of breast cancer [16]. Ethanol consumption, lack of exercise, and radiation are among well-recognized risk factors [10].

Histologically speaking, breast cancer is mostly epithelial in origin and classified into ductal and lobular. Ductal carcinoma is characterized by the glandular appearance in the form of ducts, while the lobular type had characterized by acinar configuration; however, both arise for TDLU [17-21]. These had further classified into invasive and in situ lesions. Invasive lesions are characterized by stromal invasion and breaching of the basement membrane, while in situ lesions are limited by the basement membrane. The most common type is by-far, the invasive ductal type [17-21]. Immunohistochemical evaluation of breast tissue is of diagnostic, prognostic, and therapeutic relevance. Estrogen receptor (ER) and progesterone receptor (PR) play a vital role in the management of breast cancer because they predict response to endocrine treatment; however, they have a little prognostic role regarding the fate of disease [22]. These receptors had assessed by immunohistochemistry (IHC) that has performed on paraffin tissue preparations. The cutoff value for positive and negative interpretation had regarded at 1% of nuclei [23]. The current study was aiming at highlighting the value of immunohistochemical expression of ER and PR receptors in the prognosis of Iraqi women with breast carcinoma.

MATERIALS AND METHODS

The current study was carried out at the central laboratory of Al-Diwaniyah teaching hospital and several private histopathology laboratories in Al-Diwaniyah province, the mid-Euphrates region of Iraq. The study was started in July 2019 and ended in February 2020. Thirty paraffin sections of breast carcinoma were retrieved from these laboratories. One-section had been made from each paraffin block and stained by the conventional hematoxylin and eosin (H and E) stain. Then, the sections had reviewed by two pathologists, who confirmed the diagnosis, as well as the other related histological features like grade, histological subtype, stromal invasion, and lymphatic and neural permeation. Two-further sections had made for immunohistochemical staining with ER and PR receptor stains.

The sections made were thin (5 µm) and were put on positively charged slides. The Deparaffinization step had then done using a xylene bath, three times 5 minutes for each. The rehydration step had done using descending ethanol concentrations baths 95%, 90%, and 75 %, 5 minutes for each, then followed by a distilling water bath for 5 minutes. The antigen retrieval step was carried out at microwave with EDTA buffer (pH of 8) for 20 minutes. Endogenous peroxidase was inhibited by running tissue through 6 % oxygenated water for 5 minutes. The incubation with the primary antibody has performed after washing with PBS solution for 5 minutes, and the incubation step lasted for one hour at 37 ºC. The primary antibodies used were ER (monoclonal mouse anti-human estrogen receptor α, 1D5 clone; DAKO Cytomation, Denmark) and PR (monoclonal mouse anti-human progesterone receptor, Pgr 636 clone; DAKO Cytomation, Denmark) in 1:50 dilution". The tissues were then washed with PBS/ Tween and then incubated with En Vision HPR detection system for 30 minutes at room temperature. The washing with water was then carried out and then followed by signal visualization using 3-3' diaminobenzidine DAB. Hematoxylin was then used for nuclei counterstaining. The dehydration step has then been completed, with ascending ethanol concentration baths followed by the clearing step and mounting using Canada balsam and covered by a coverslip. With each run of staining control, the positive and technical-negative controls had included [24-26]. The stain evaluation had done according to the Allred score depending on both the proportion and intensity of stain [24].

RESULTS

The Clinicopathological features of cases with breast carcinoma enrolled in the present study are shown in table 1. The predominant histopathological type was invasive ductal carcinoma with some cases of invasive lobular carcinoma, 23 (76.7 %) versus 7 (23.3 %), respectively, table 1. The most frequent grade was moderate grade accounting for 19 cases (63.3%), followed by high-grade, which accounted for 6-cases (20.0 %), and finally by low-grade cases, which had observed in 5-cases (16.7 %). Low grade denotes well-differentiated mammary carcinoma with very-close morphology to the original glandular and stromal appearance of normal breast tissue. The moderate-grade indicates the presence of some areas that lack the conventional stromal and glandular morphology of normal mammary gland, while high-grade described cases in which there is a poor-differentiation with predominant lack of the normal glandular and stromal appearance of normal mammary tissue.

The reports retrieved from the laboratory also contained information about the clinical staging of the disease. Accordingly, there were 5 cases with stage II, 18-cases with stage III, and 7-cases of stage IV disease. The word "staging" means the extent of the disease. Therefore, stage II disease indicates less spread of tumor while stage four disease denoted distant metastasis. The permeation of lymphatic channels (by malignant cells) had observed in a single case.

The immunohistochemical expression of ER and PR receptors shown in table 2. Positive ER only cases were 3 (10.0 %), positive PR only cases were 4 (13.3 %), and those were positive for both ER and PR were 15 (50.0 %). The samples that were negative for both ER and PR accounted for 8 (26.7 %). Total cases expressing ER accounted for 18 (60.0 %), and those PR-express, had accounted for 19 (63.3 %).

Table 1: Clinicopathological features of cases with breast carcinoma

Characteristic	Number of cases	%
Histological subtype		
Ductal carcinoma	23	76.7
Lobular carcinoma	7	23.3
Grade		
Low grade	5	16.7

Moderate grade	19	63.3
High grade	6	20.0
Stage		
II	5	16.7
III	18	60.0
IV	7	23.3
Lymphatic permeation	3	10.0

Table 2: Immunohistochemical expression of ER and PR receptors

Immunohistochemistry	Number of cases	%
Positive ER only	3	10.0
Positive PR only	4	13.3
Positive ER and PR	15	50.0
Negative ER and PR	8	26.7
Total ER cases	18	60
Total PR cases	19	63.3

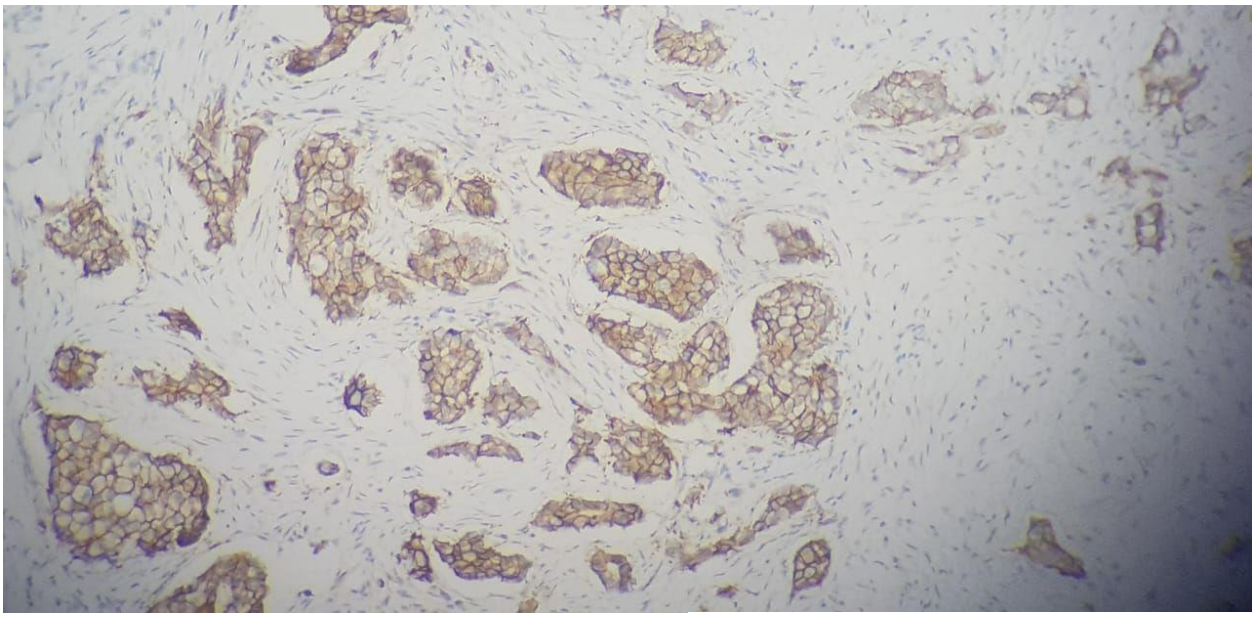

Figure 1: Histological section through breast biopsy showing infiltrative ductal carcinoma with (estrogen receptor) ER-positive immunohistochemical stain. There is an invasion of stromal tissue by moderately differentiated glandular malignant epithelial structures that show prominent cytoplasmic and membranous ER staining of nearly all malignant epithelial shown in the section. X10.

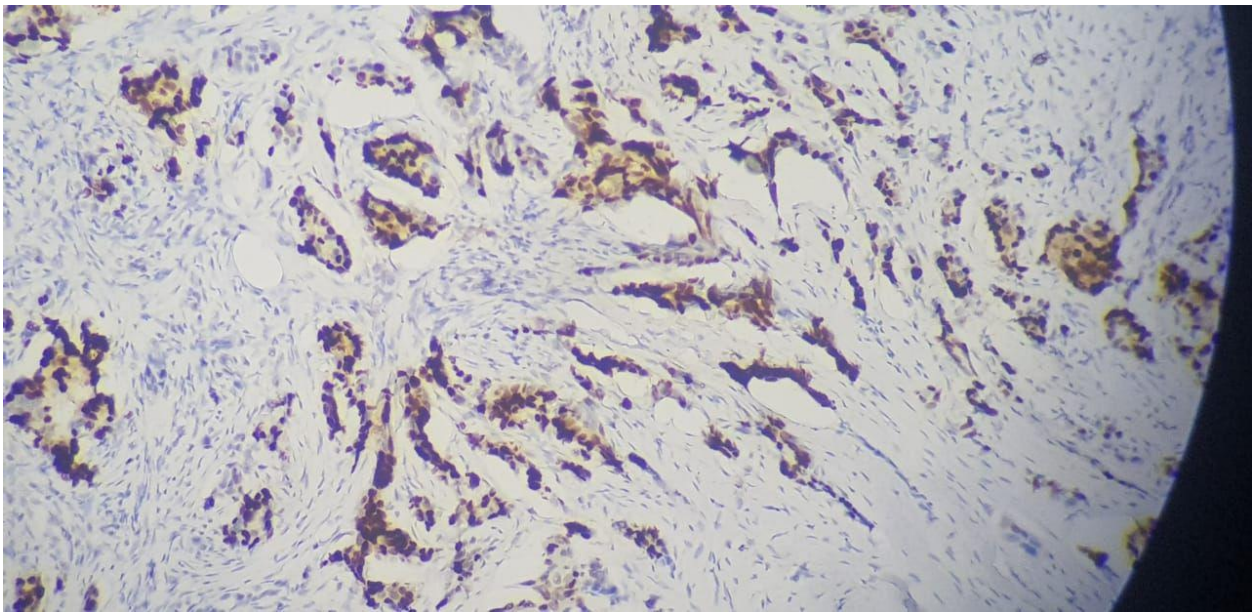

Figure 2: Histological section through breast biopsy showing infiltrative ductal carcinoma with (progesterone receptor) PR positive immunohistochemical stain. There is an invasion of stromal tissue by poorly differentiated glandular malignant epithelial structures that show prominent cytoplasmic and membranous PR staining of nearly all malignant epithelial shown in the section. X10.

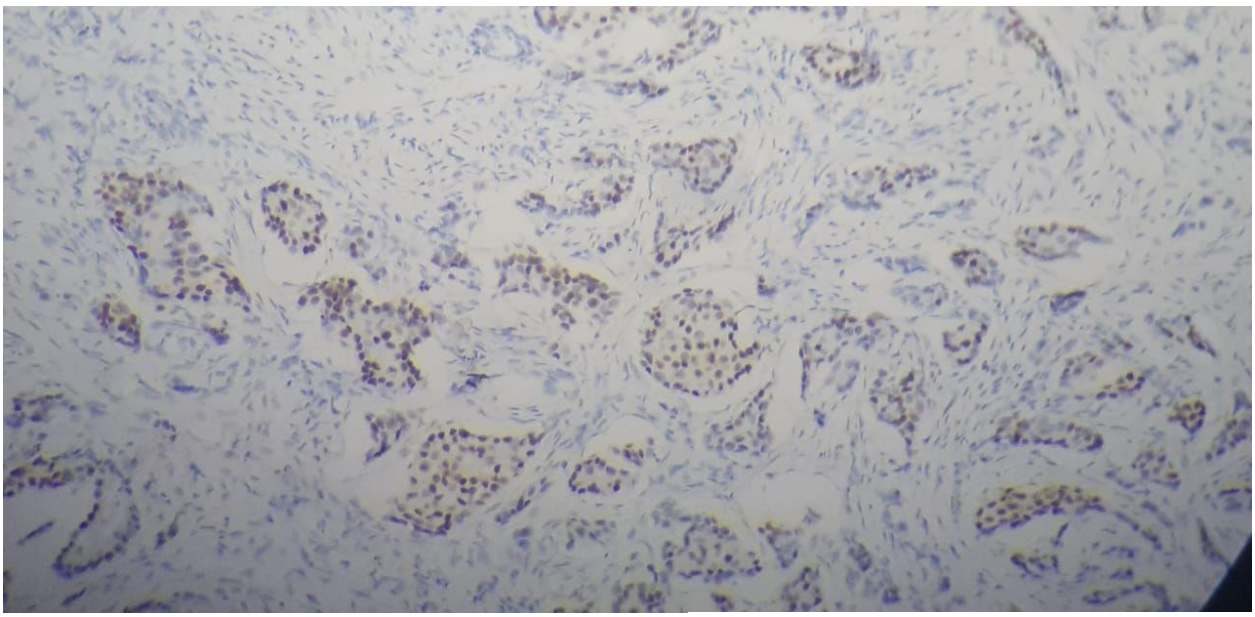

Figure 3: Histological section through breast biopsy showing infiltrative lobular carcinoma with (estrogen receptor) ER-positive immunohistochemical stain. There is an invasion of stromal tissue by lobular monomorphic glandular malignant epithelial structures that show prominent nuclear, cytoplasmic, and membranous ER staining of nearly all malignant epithelial shown in the section. X10.

DISCUSSION

Hormonal therapy is nowadays a corner step in the management of breast cancer; therefore, it is of prime importance to evaluate hormone receptor status, namely ER and PR of every case of breast cancer. In the present study, 60 % of the samples showed positive expression of ER, and 63.3 % of the cases, showed positive expression of PR receptors. These results are similar to results obtained by several other authors [24, 27]; however, they are inconsistent with results obtained by many previous reports [28-30]. One of the principal findings in this work is the lack of homogeneity of receptor immunohistochemical-staining. Some cases showed a high proportion of cells with intense staining pattern; others showed mild faint staining with little-proportion, and others showed intense staining despite low proportional staining. These findings may explain the lack of consistent response to hormonal therapy despite positivity for ER and ER receptors. It had estimated that 30-40 % of cases of breast cancer do not respond to hormonal treatment despite being positive for both ER and PR receptors [24, 31]. The heterogeneity was more marked for progesterone receptors, a finding that is in agreement with previous reports (24, 30) and may partially explain the high proportion of false-negative results in determining PR status in breast cancer tissue sections [24, 32]. In the current study, also we observed that ductal carcinoma expressed ER in less proportion than lobular carcinoma, an observation that is supported by previous reports [24, 33]. In the current study, 26.7 % of cases were negative for both ER and PR receptors.

This phenotype of ER-/PR- has been said to account for approximately 25 % of breast cancer cases [34]. Some of the samples had ER + phenotype or PR + phenotype. Previous reports also identified ER+ only phenotype or PR+ only phenotype [24]. The presence of PR receptors requires the existence of ER receptors from a physiologic point of view; therefore, it is likely that cases which are ER-/PR+ in the current study are the results of the low level of ER that cannot be detected by conventional immunohistochemical staining [34]. Indeed, the presence of only one receptor positivity is of poor prognosis for hormonal therapy since cases with both receptor positivity respond to hormonal therapy; two times better than those with single receptor positivity [24, 35]. Changes in hormone receptor expression have noted in cases treated with the hormonal therapy tamoxifen, and these changes may lead to diminished expression of both receptors making the tumor more resistant to hormone therapy [24, 35]. In conclusion, the immunohistochemical expression of ER and PR receptors is heterogeneous concerning the proportion and intensity of staining, and this may explain the variation of breast cancer cases in response to hormonal therapy.

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