

Immunohistochemical expression of ER/PR in cytological and histopathological preparation of infiltrative ductal carcinoma of breast: comparison clinicopathological study

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Summary:

Background: Breast cancer is the leading cause of cancer death among women worldwide and in Iraq.

Objectives: To evaluate the reliability of the immunocytochemical method in breast carcinoma aspirates with those obtained by immunohistochemistry of the corresponding excised breast tissue sections.

Patients and methods: This is a prospective study. A total of (41) patient complain from malignant breast lump and (10) cases with benign mass (fibro adenoma) visited the Oncology Teaching Hospital in the Medical City in Baghdad during the period from the first of April 2012 to the end of December 2012. FNA sample taken for routine cytopathological and immunocytochemical study and the patient where followed up and immunohistological study was done for mastectomy or tissue biopsy samples.

Results: The diagnostic sensitivity, specificity, PPV, and NPV of estrogen receptor ICC staining as Compared to the ER IHC were 92%, 62%, 50% and 95% respectively. The overall accuracy was 59.2%. The diagnostic sensitivity, specificity, PPV, and NPV of progesterone receptor ICC staining as Compared to the PR IHC were 93%, 62%, 58%, and 94% respectively. The overall accuracy was 73.2%

Conclusion: There was no statistically significant correlation between ER, PR expression in both ICC and IHC

Key words: immunocytochemical, immunohistochemical, estrogen, progesterone.

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Introduction:

Carcinoma of the breast is the most common non-skin malignancy in women. (1). In North America and Europe showed a 5-10% decline in the incidence of breast cancer following reductions in menopause hormone therapy (HT) use after 2002 and the technical improvements and the increased effectiveness of breast cancer screening and detection (2) Breast screening should be performed in the presence of adequate facilities for diagnosis, including: Fine needle aspiration cytology and/or excision biopsy; ultrasound; mammography, if available, (mammography may be negative in a woman with breast cancer, especially if she is under the age of 50) (3) Breast cancer in the Middle-East occurs in relatively young women and frequently presents as advanced disease. (4) In Iraq, breast cancer is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers according to the latest Iraqi Cancer Registry (5). 70-80% of breast carcinoma expresses ER and PR. Estrogen receptors are over-expressed in around 70% of breast cancer cases, referred to as ER-positive (6), ER-positive and ER-negative carcinomas show striking differences with regard to patient characteristics, pathologic features, treatment response, and

outcome (1). Recently the possibility of using cytological materials, not only for morphological diagnosis but also for biologic characterization of the tumors (7) Relatively few studies have investigated the correlation between hormone receptor assessment by ICC on preoperative fine needle aspiration cytology with the results determined by IHC of the corresponding surgical samples [8,9,10] This study was performed by using freshly prepared cytological samples.

Patients and methods:

This is a prospective study, during the period from April 2012 to December 2012, 41 patients complaining of apparent breast lumps visited the *Oncology Teaching Hospital in the Medical City in Baghdad*. The age of the women ranged between (33-80 years). Only those in whom pathological examination revealed malignant changes were included in this study. Fine needle aspiration done from malignant breast mass and cytological smear done for both conventional cytological methods using Papanicolaou stain and ICC staining with anti-estrogen/ progesterone monoclonal antibody. Follow up of patients after mastectomy operation and IHC staining using ER/PR done on slides prepared from formalin fixed paraffin embedded tissue blocks of same patient. The immunostaining methods used for cytology specimens are identical to those used for histologic studies, including the

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antigen retrieval steps (12). the immunostaining technique was done at the Iraqi National Cancer Research Center and at the Teaching Laboratories of Baghdad Medical City teaching hospital. This technique basically uses an unlabeled primary antibody, which was mouse monoclonal antibody purchased from DAKO {Code No of the kits was M7047 for ER and M3569 for PR}, it binds to its corresponding antigen, followed by a biotinylated secondary antibody to which the avidin-biotin complex attaches. If the antigen is present in the sample there will be antigen-antibody interaction and enzymatic reaction that can be detected by the chromogen, diaminobenzidine(DAB) which can be visualized by light microscope. Negative controls were obtained by replacing the primary antibody with buffer saline and Positive controls by using breast carcinoma sections which are known to express ER,PR.

Statistical Analysis:

Statistical analysis was performed with SPSS V. 17(statistical package for social sciences) and also Excel 2010 programs using Chi square test and T test. The values were considered statistically significant when P value is less than 0.05.

Results:

In this prospective study; all of the patients were females, the mean age of the patients was (52.12 years). With a range of (33-80) year The scoring was performed according to the Allred Score System(13) which includes proportion score(PS) and intensity score(IS) to get the total score {total score (TS) =sum of PS and IS}. A positive result for both ER and PR is defined as TS equal or more than

3, whereas those with a TS less than 3 were considered negative cases .

ER and PR coexpression in ICC: Eleven cases show positive expression for both ER and PR, 1 case was ER positive and PR negative, 4 cases were ER negative PR positive while 25 cases were both ER and PR negative.

ER and PR coexpression in IHC: Twenty one case show positive expression for both ER and PR, 1 case was ER positive and PR negative, 3 cases were ER negative PR positive while 16 cases were both ER and PR negative.

ER IHC status versus ER ICC status: The ICC results were then compared with those of IHC of the corresponding excised surgical specimens. Among the 12 estrogen receptor ICC positive cases, 11 cases were positive by IHC (true positive) and 1 case was negative by IHC (false positive). Among the 29 estrogen receptor negative cases 18 cases were negative by IHC (true negative) and 11 cases were positive by IHC (false negative) as shown in figure 1. Compared to the IHC, the diagnostic sensitivity, specificity, PPV, and NPV of estrogen receptor immunocytochemical staining were 92%, 62%, 50% and 95% respectively. The overall accuracy was 59.2%

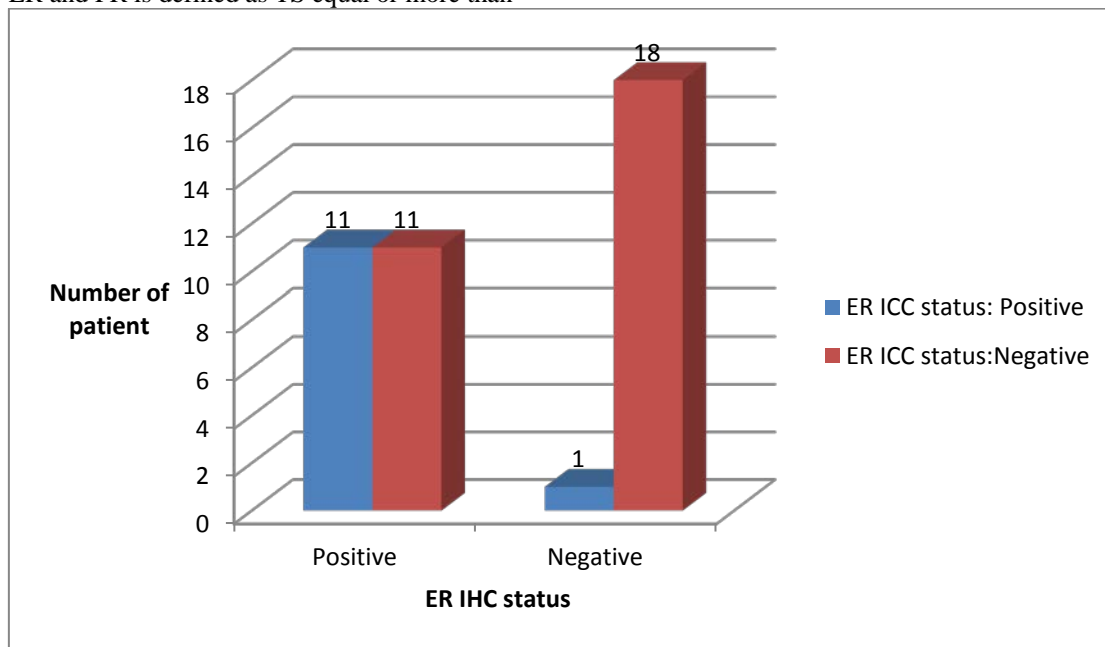


Figure 1: ER IHC status versus ER ICC status

PR IHC status versus PR ICC status: Among the 15 progesterone receptor ICC positive cases, 14 cases were positive by IHC (true positive) and 1 case was negative by IHC (false positive). Among the 26 progesterone receptor negative cases, 16 cases were

negative by IHC (true negative) and 10 cases were positive by IHC (false negative) as shown in figure 2. In comparison to the IHC, the diagnostic sensitivity, specificity, PPV, and NPV of progesterone receptor immunocytochemical staining

were 93%, 62%, 58%, and 94% respectively. The overall accuracy was 73.2%

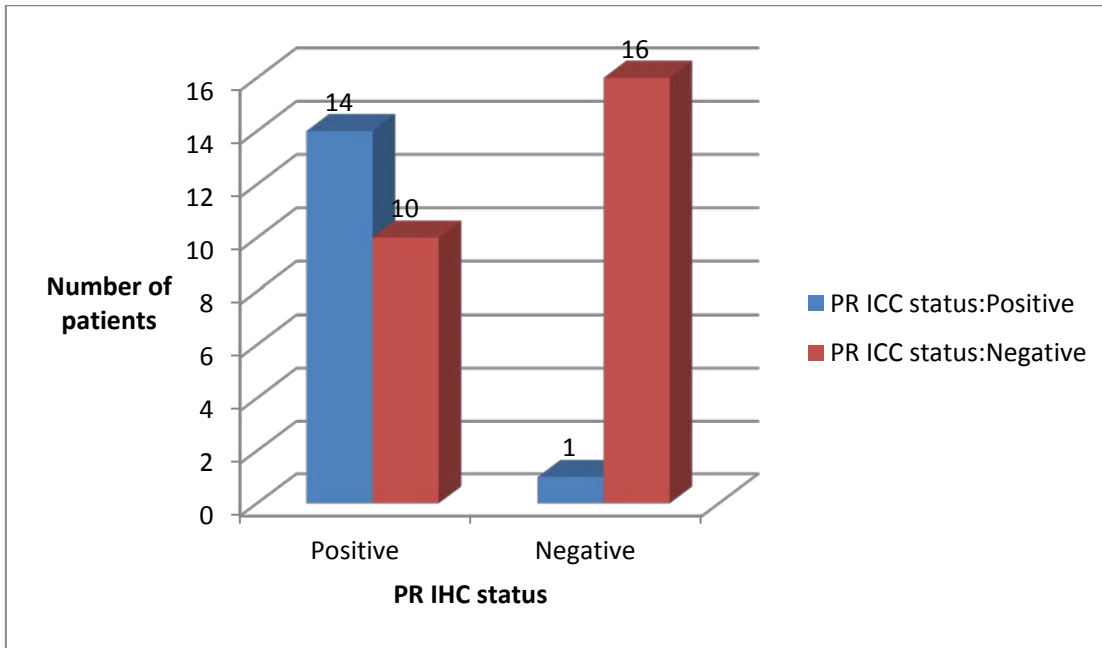
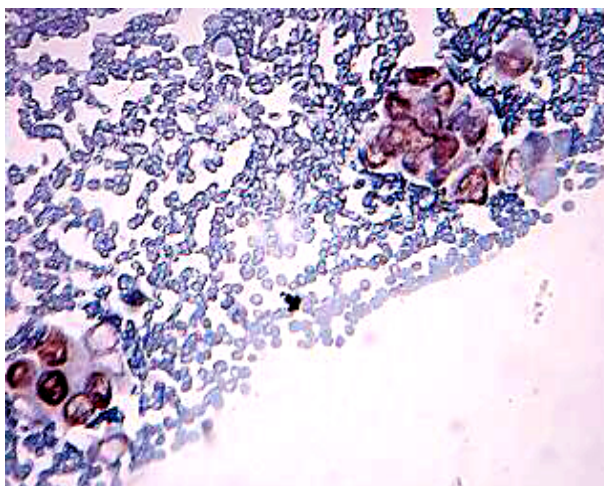
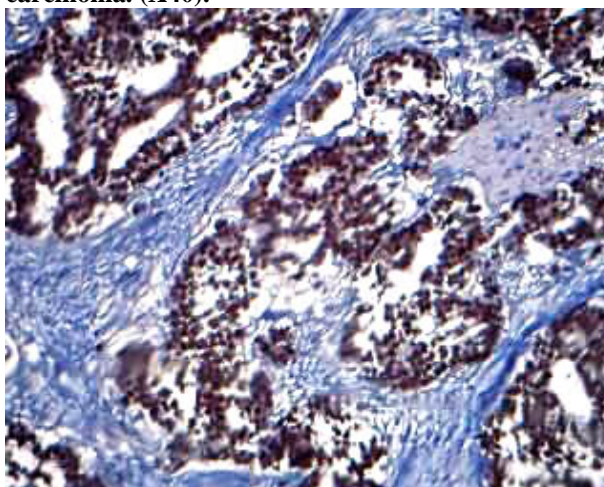


Figure 2: PR IHC status versus PR ICC status



Photomicrograph 1: positive ICC expression of PR (nuclear expression) in FNA of ductal breast carcinoma. (X40).



Photomicrograph 2: positive IHC expression of PR (nuclear expression) in histopathological sample of ductal breast carcinoma. (X20).

Discussion:

In this study the age of the patient was comparable to that reported by other Iraqi workers(14,15,16)and to Jordanian study(17) but the age range was lower than that reported by American studies(17,18) how reported mean age(59.8 , 61.6) year respectively. This difference can be attributed to the variation in population structure, as life expectancy in western countries is longer than in Iraq, lack of routine checkup for Iraqi patient and difference in the sample size.

ER marker expression: ER ICC, In this study 29.3% show a positive immunocytochemical expression while Hiba Ahmed show higher percent 57.5% of cases (15). Other study done by Nesreen and Neeven the total number of estrogen receptor positive cases was 69 cases (76.7%), this author have used the destained slides(11), this differences could be explained by many factors like sized sample, technical factors as will be mentioned latter . In our study there was no statistically significant correlation between ER ICC expression and age of patient with P value 0.5 (more than 0.05).

ER IHC : In the present study 53.7% were positive by ER IHC while in Ahmed Raji K study the ER was positive in 9.19% (20). Statistically there were no significant correlation between age and the ER IHC staining (p = 0.76) (N.S).

PR marker expression: PR ICC, In this study, 36.6% show a positive immunocytochemical expression while HibaAhmed show 35% of cases (15) While in Nesreen and Neeven study(11) the total number of progesterone receptor positive cases were 62 cases (68.9%). In our study there was no statistically significant correlation between PR ICC expression and age of patient with P value =1 (more than 0.05)

PR IHC: In the present study 58.5% were positive by PR IHC while in Ahmed Raji K, the PR was

positive in 29.88% (20). Statistically there were no significant correlation between age and the PR IHC staining ($p = 0.35$) (N.S). In the present study, the concordance results (Accuracy) of estrogen (59.2%) with 93% sensitivity and 62% specificity and the concordance results of progesterone receptor (73.2%) with 93% sensitivity and 62% specificity, our results were higher than Radhika and Prayaga study who recorded 50% concordance rate for estrogen receptor and 29% concordance rate for progesterone receptor immunocytochemistry with 33% sensitivity and 75% specificity for estrogen receptor; and 25% sensitivity and 33% specificity for progesterone receptor immunocytochemistry. They attributed the cause of their poor results to technical factors such as using poor buffer and improper antigen retrieval due to financial cause (8). While Nesreen and Naveen (11) show the concordance results of both estrogen (91.1%) and progesterone receptor (88.9%) were in line with the concordance between cytology and histology (84%) for estrogen receptor and (90%) for progesterone receptor in the study done by Malaviya et al. (21) these differences could be attributed to differences in sample size and other obstacles and it's important to be noticed that most of these researches done at destaining procedures. Nizzoli et al. study (9) found 89% agreement for estrogen receptor immunocytochemistry and 78% for progesterone receptor when compared with the IHC results. In Krishnamurthy et al., study (22) studied the hormone receptor assessment by ICC on archival Papanicolaou stained smears and concluded that the negative results were less reliable as an indicator of the true hormone receptor status than the positive results. This result agreed with our study where most of discordant cases were among the negative results where it was 11 cases (37.8%) out of 29 negative estrogen receptor results and 10 cases (38.5%) out of the negative progesterone receptor results. While the positive results showed only 1 false positive result (8.3%) among the 12 estrogen positive cases and one false positive result (6.7%) among the 15 progesterone positive cases. ICC is a well-accepted technique to determine the ER and PR status in the treatment of breast cancer. The results of both ER and PR expression in these previous studies were slightly higher than the results of our study; this may result from the fact that the sample in our study is not so large and so may be not representative of true population with breast carcinoma, also due to different other factors regarding geographic, ethnic differences.

Conclusion:

There was no statistically significant correlation between ER, PR expression in both ICC and IHC.

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