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**ORIGINAL RESEARCH ARTICLE** **OPEN ACCESS**

# UTILITY OF ESTROGEN RECEPTOR ALPHA (ERα), PROGESTERONE RECEPTOR (PR), AND HER2 EXPRESSION IN PRIMARY BREAST CARCINOMA IN IRAQI WOMEN

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**ABSTRACT**

**Background:** Immunohistochemical markers can be used to classify breast cancer into distinct biological subtypes that behave differently. The Hormone receptors (estrogen receptor (ER) and progesterone receptor (PR)), and human epidermal growth factor receptor-2 (HER2) profile of breast carcinoma have a significant role in determining patient's prognosis, in addition to early detection of cancer, planning treatment and monitoring response to treatment. Increase use of tumor markers represents a shift in understanding of the basic biology of breast cancer, and treatment modules. The aim of this study was to assess immunohistochemical (IHC) profiles (Estrogen receptor (ERα), progesterone receptor (PR) and HER2 of primary breast carcinomas in Iraqi women, and their relation to different clinicopathological parameters.
**Materials & Methods:** Breast tissue samples were assessed using tumor biomarkers for hormone receptor (HR) and human epidermal growth factor receptor-2 (HER2) expression. Histologic data was collected for each case, including tumor size (cm), tumor grade, stage, lymph node status, and IHC panel (ERα, PR, HER2).
**Results:** Of the 47 carcinomas studied, 63.8 % were positive for ERα, 23.4% positivity for PR, and 23.4% were ERα positive/PR positive (ER+/PR+). For 47 cases with HER2 IHC, 25.5 % were positive. ER and PR association with clinicopathological parameters was not significant. The positive expression of HER2 of tumors was significant association with histological grade.
**Conclusions:** Profiles for ER, PR IHC were not significantly associated with stage & grade of the tumor, and their expression may be independent from the grade and stage of tumor. Further investigation is warranted to assess reproducibility of technique and investigate clinical implications of PR status in primary breast carcinoma.



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## INTRODUCTION

Breast cancer is the most common form of neoplasm and is the second most common cause of cancer-related deaths among women worldwide. In spite of the wide research in this area, still breast cancer is a heterogeneous disease, with a long natural history, its behavior remains not clear and some patients have a higher risk of recurrence than others. Breast cancer mortality has been declining because of advances in the use of adjuvant therapies (Tremont, 2017 and Cureton, 2015).

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Tumor markers, hormone receptors (HRs) and proto-oncogene (HER2) profile of a female breast carcinoma plays a significant role as a predictive marker in patient prognosis and management. In addition to factors such as age, tumor size, lymph node involvement, histologic type, and tumor grade, the hormone receptor and HER2 status at the time of initial diagnosis has been established as a clinically useful, standard-of-care parameter in determining treatment options and subsequent patient response (Kinsella, 2012). The 2 major ERs are ERα and ERβ. Most of breast tumors express ERα. Hormone receptors (ER & PR) and human epidermal growth factor receptor 2 (HER2) are recommended for early detection of cancer, to plan treatment, monitoring response to treatment, and determining patient's prognosis" (Lyndsay Harris, 2007).

In addition to being a prognostic factor itself, ER positive status is more likely to be found in well-differentiated tumors, older women, and those with a lower mitotic number and in diploid ones. ER positive tumors are less likely to exhibit a mutation, loss or amplification of breast cancer related genes (p53, HER2, EGFR) (Sarkar, 2008). A study of risk partitioning by Essermanand coworkers (Esserman, 2011). showed that adjuvant naïve node-negative outcome risk was primarily partitioned by tumor receptor status and grade but not tumor size. HR-positive and HER2-negative (HRpos) risk was partitioned by tumor grade; low grade cases have very low early risk but a 20% fall-off in Disease-specific survival (DSS) 10 or more years after diagnosis. Higher grade HRpos cases have risk over >20 years. Triple-negative (Tneg) and HER2-positive (HER2pos) cases DSS events occurred primarily within the first 5 years. Among node-positive cases, only low grade conferred late risk, suggesting that proliferative gene signatures that identify proliferation would be important for predicting early but not late recurrence.HR+/HER2- breast cancers, the subtype with the best prognosis, were the most common for all races/ethnicities with highest rates among white women. Triple-negative breast cancers, the subtype with the worst prognosis, were highest among non-Hispanic black women (Esserman, 2011 and Kohler, 2015). Remarkable insights into tumour biology in the last century have been the catalyst to the meteoric rise of medical oncology (Lakhtakia, 2005). The relationship of HER2 and response to endocrine therapy is interesting. In HER2 positive metastatic disease response rates to tamoxifen are lower than HER2 negative patients. In adjuvant setting, HER2 positive tumors tend to have shorter DFS/OS when treated with tamoxifen. Recent trials using Herceptin as a form of molecular targeted therapy in HER2 positive patients have shown promising results in the metastatic setting (Huang, 2005).

## MATERIALS AND METHODS

**Case Selection:** Approval for use of human subjects was obtained from Tikrit University Review Board. The study was done at Cardiff School of Bioscience laboratory – UK. A total of 47 infiltrative ductal carcinoma and 7 benign cases, diagnosed by surgical resection specimen biopsy, and identified through retrospective review of surgical pathology report databases from the archives of Histopathology and Cytology Unit in Tikrit Teaching Hospital-Salah Al-Din-Iraq. Data including patient age, tumor size, histologic grade, and presence or absence of metastases, and pathological stage was obtained for each patient. All surgical specimens had been evaluated by faculty surgical pathologists at College of Medicine - Aliraqia University and specialist pathologists at Cardiff School of Bioscience laboratory, UK. Tumors were graded according to (Scarff Bloom Richardson), (combines nuclear grade, tubule formation, and mitotic rate, with I being well-differentiated, and III being poorly differentiated for each. The scores were then summed to getthe histologic grade; a score of 3 to 5 = well-differentiated (grade I), 6 to 7= moderately differentiated (grade II), and 8 to 9 = poorly differentiated (grade III).

**TMA construction:** For each case of study cohort, an initial hematoxylin and eosin-stained control section was reviewed to confirm an adequate tissue in donor block for transfer to the tissue microarray (TMA) block and to select and mark the location points for cores to be taken. Beecher TMA instrument (Beecher Instrument, Sun Prairie, WI 53590) was used to remove 2 cores of 0.6 mm from each donor block and transferred them to a recipient block. Cores were arranged in sectors, each containing 12 rows with 12 cores per row, and the distance between each two cores 1mm and each two rows 1mm.

**Immunohistochemistry and Scoring for ERα, PR and Her2/*neu***

TMA block was cut at a thickness of 5µm on a microtome cutter (Leica RM2135). Sections were placed on poly-L-lysine (PLL) coated slides (polysine, Thermo Fisher) and heated at 58°C for 24 hours after that the melting paraffin wax was added on the top of TMA section to prevent loss of cores. Slides were deparaffinized and rehydrated in graded alcohols, heat-induced epitope retrieval were done by immersing them in a 0.01-mol/L concentration of citrate buffer (pH 6.0) preheated to more than 90°C and left for 20 minutes, followed by 20-minutes cool down period at 25-28°C. IHC was performed, following optimized epitope retrieval, using mouse monoclonal antibodies: ERα (1D5) (1:50), and PR (PgR 636) (1:400) (DAKO). Polyclonal HER2 antibody in the Herceptin kit (HercepTest, DAKO) was used according to the manufacturer's instructions. The slides are counterstained in hematoxylin, and finally coverslipped. Positive control of known positive tissues (breast) and negative controls with primary antibody replaced with TBS were run with the patient slides. The stained slides were scanned and quantified by consultant pathologist, using light microscopy. ERα and PR were brown nuclear staining in tumor cells. Two considerations were taken, theproportion of positive cells (scored on a scale of 0-5) and staining intensity (scored on a scale of 0-3). Every tumor was given a score which represents the outcome of the summation of the intensity of the staining(intensity of score IS) (no staining = 0; weak staining = +1; intermediate staining= +2; strong staining = +3) with the percentage of stained cells(proportion score PS) (score 0 = no stain, score 1= less than 1%, score 2; > 1-10%, score 3; > 10-33%, score 4; > 33-66% and score5; 66-100%). The proportion and intensity were then summed to gain total scores of 0 or 2 through 8. A score of 0 -2 was regarded as negative, while 3-8 as positive. The maximum score according to this system was8 (Zhou, 2014). Scoring of HER2;Cut off values of HER2 receptor were scored according to DAKO scoring system, using the following categories: 0, negative result or membrane staining in <10% of the tumor cells; 1+; weak and incomplete brown membrane staining in >10% of the tumor cells; 2+; weak or moderate, complete brown membrane staining in >10% of the tumor cells; 3+; strong complete brown membrane staining in >10% of the tumor cells). Score +1 considered negative.

**Statistical Analysis**

Fisher's Exact test, and ANOVA were used. $P$ value < 0.05 was considered as significant.

## RESULTS

ERα and PR were brown nuclear staining, HER2 was brown membrane staining (Figure 1). This study classified the primary breast cancer into six groups, defined by expression of the three markers ERα, PR and HER2 ; ERα positive/PR positive (11/47, 23.40%), ERα negative/PR negative (17/47, 36.17%), ERα positive/PR negative (19/47, 40.42%), ERα

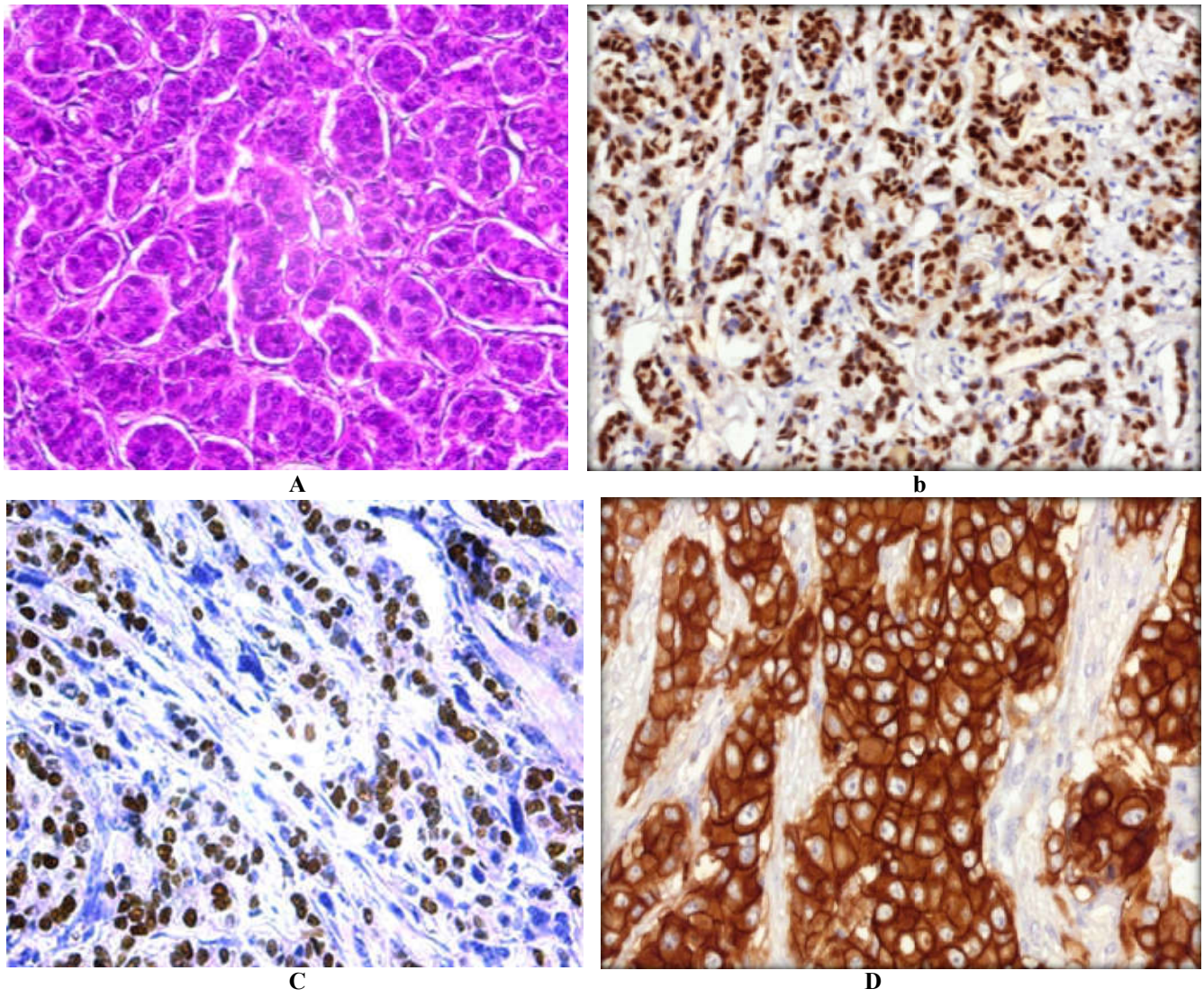


**Figure 1. ERα, PR, and HER2 IHC Profile of infiltrative ductal carcinoma (IDC) of breast, poorly differentiated (Scarff Bloom Richardson grade III).**
**A; Infltrative ductal carcinoma. Poorly differentiated (grade III). (40x, H&E).**
**B; Strong positive expression of ERα (brown nuclei), (IS + 3 & PS 5, Total score = 8) (40x).**
**C; Strong positive expression of PR (brown nuclei), (IS + 3 & PS 4, Total score = 7) (40x).**
**D; Strong positive expression of HER2/neu, brown membranous stain, score 3+, (complete membranous reactivity in > 10% of tumor cells) (40x)**

**Table 1. Status of ERα PR& HER2 in 47 Cases of Infiltrating Ductal Carcinoma of Breast**

| Hormonal receptors expression | Number of IDC - (%) |
|---|---|
| $ER^+$ | 30 (63.82) |
| PR+ | 11 (23.40) |
| $ER^+/PR^+$ | 11 (23.40) |
| $ER^+/PR^-$ | 19 (40.42%) |
| $ER^-/PR^+$ | 0 |
| $ER^-/PR^-$ | 17 (36.17%) |
| HER-2 + | 12 (25.53) |
| $ER^-/PR^-$/ HER2/neu- | 12 (25.53) |

negative/PR positive (0%), HER2 positive (12/47, 25.53%), ERα negative/PR negative/ HER2 negative (Triple Negative Breast Cancer (TNBC)) (12/47, 25.53%,) (Table -1). All PR positive tumors were positive for ERα and all ERα negative tumors were negative for PR.

**Association of ERα, PR and HER-2 expression with patient's age:** There was indirect correlation between hormone receptors and HER2with patient's age, ($p > 0.05$), (Table 2).

**B- Association of ERα,PR and HER 2 expression with clinicopathological features of breast cancer**

The analysis of ERα,PR and HER-2 expression with clinicopathological features of breast cancer demonstrated that no significant association between ERα,PR and HER-2 expression with tumor size, lymph node status and pathological stages, as well no significant association between histological grade with ERα, PR expression but significant with HER-2 level, Table 3

**Table 2. Association of ERα, PR& HER2 Expression with Age**

| ERα | Number of IDC - (%) | Age | *P* value |
|---|---|---|---|
| | | Mean ± SD | |
| Negative | 17 (36.17) | 48.7±12.8 | 0.3 |
| Weak positive | 7 (14.89) | 46.3±9.6 | |
| Moderate positive | 14 (29.78) | 52.8±12.5 | |
| Strong positive | 9 (19.14) | 54.9±9.4 | |
| Total | 47 (100) | 50.7±11.8 | |
| PR | Number of IDC - (%) | Age | *P* value |
| | | Mean ± SD | |
| Negative | 36 (76.59) | 50.9±12.7 | 0.3 |
| Weak positive | 6 (12.76) | 44.7±5.7 | |
| Moderate positive | 4 (8.51) | 55.0±7.3 | |
| Strong positive | 1 (2.12) | 65.0 | |
| Total | 47 (100) | 50.7±11.8 | |
| HER-2 | Number of IDC - (%) | Age | *P* value |
| | | Mean ± SD | |
| Negative | 35 (74.46) | 51±12.6 | 0.7 |
| Positive | 12 (25.53) | 49.2±9.4 | |
| Total | 47 (100) | 50.7±11.8 | |

**Table 3. Association of ERα,PR and HER 2 Expression with Clinicopathological Features of Breast Cancer**

| Pathological Features | Number of IDC - (%) | ER Expression Number of IDC - (%) | | PR Expression Number of IDC - (%) | | HER-2 Expression Number of IDC - (%) | |
|---|---|---|---|---|---|---|---|
| | | $ER^+$ | $ER^-$ | $PR^+$ | $PR^-$ | HER-$2^+$ | HER-$2^-$ |
| Tumor size cm | | | | | | | |
| >2cm | 44 (93.61) | 30 (68.18) | 14 (31.81) | 11 (25) | 33 (75) | 11 (25) | 33 (75) |
| ≤ 2cm | 3 (6.38) | 0 | 3 (100) | 0 | 3 (100) | 1 (33.33) | 2 (66.66) |
| *P* value | | 0.2 | | 0.3 | | 1.0 | |
| Nodal status | | | | | | | |
| Positive | 32 (68.08) | 22 (68.75) | 10 (31.25) | 6 (18.75) | 26 (81.25) | 6 (18.75) | 26 (81.25) |
| Negative | 15 (31.91) | 8 (53.33) | 7 (46.66) | 5 (33.33) | 10 (66.66) | 6 (40) | 9 (60) |
| *P* value | | 0.7 | | 0.3 | | 1.0 | |
| Histological grade | | | | | | | |
| Grade I | 6 (12.76) | 2 (33.33) | 4 (66.66) | 0 | 6 (100) | 2 (33.33) | 4 (66.66) |
| Grade II | 32 (68.08) | 21 (65.62) | 11 (34.37) | 8 (25) | 24 (75) | 5 (15.62) | 27 (84.37) |
| Grade III | 9 (19.14) | 7 (77.77) | 2 (22.22) | 3 (33.33) | 6 (66.66) | 5 (55.55) | 4 (44.44) |
| *P* value | | 0.4 | | 0.8 | | 0.03* | |
| Pathological stage | | | | | | | |
| I | 3 (6.38) | 0 | 3 (100) | 0 | 3 (100) | 1 (33.33) | 2 (66.66) |
| IIA | 9 (19.14) | 6 (66.66) | 3 (33.33) | 3 (33.33) | 6 (66.66) | 3 (33.33) | 6 (66.66) |
| IIB | 11 (23.40) | 9 (81.81) | 2 (18.18) | 3 (27.27) | 8 (72.82) | 2 (18.18) | 9 (81.81) |
| IIIA | 17 (36.17) | 10 (58.82) | 7 (41.17) | 2 (11.76) | 15 (88.23) | 5 (29.41) | 12 (70..58) |
| IIIB | 7 (14.89) | 5 (71.42) | 2 (28.57) | 3 (42.85) | 4 (57.14) | 1 (14.28) | 6 (85.71) |
| *P* value | | 0.3 | | 0.3 | | 0.8 | |

* Significant association, $p<0.005$

## DISCUSSION

In addition to the known standard parameters as tumor size, lymph node status, histologic type, tumor stage and grade, the hormone receptor (ER & PR) and human epidermal growth factor receptor 2 (HER2) status of a primary breast carcinoma have clinical importance in assessment of patient prognosis and treatment options. Estrogen and progesterone are the primary regulators of breast tissue growth and differentiation. They exert their effects through binding to specific nuclear receptors, the estrogen and progesterone receptors (ERs & PRs). Once activated, the receptors exhibit transcriptional and membrane localized signaling activities. The 2 major ERs are ERα and ERβ. Most of breast tumors (70%) expresses ERα (Tremont, 2017). Studies revealed that patients with ER+/PR + tumors had a better prognosis than patients with ER +/PR- tumors, who in turn had a better prognosis than patients with ER -/ PR- tumors. However, the recurrence rate for ER positive lymph node negative breast cancer, exceeds those of other clinical groups of patients (Triple-negative patients and HER2 positive patients), whom remain free of the disease for 5 years (Zhang, 2013 and Alwan, 2017).

The results of this study revealed that the majority of infiltrative ductal carcinoma of breast (63.8%) was ERα positive. PRexpression was positive in only 23.4%. HER2 expression was positive in 25.53% of the cases.Lee and coworkers [13] showed ER positive expression in 203 out of 319 cases (63.6%). Similar results were reported by tworecent studies from Iraq for the positive IHC expression of ERand HER2 in primary infiltrating ductal carcinoma but not for PR expression. They reported higher percent for PR expression (64%) compared to our (23.40%) (Alwan, 2017& 2017). Many factors (genetic& environmental),canactivate or suppress ER/PR expression. Laboratory factors, such as the immunohistochemical processing and heat induced antigen retrieval (HIAR)technique, can modified several localization of steroid receptors in breast cancer, and the pretreatment steps (tissue processing) (fixation time and age of paraffin blocks)all may affect the sensitivity of steroid receptors (Lee, 2007). In addition to the variables among different studies like grade and stage of the tumor that may affect the results of positive expression. Study from Iran on breast cancer revealed that the expression of ER+/PR+ (55.8%), ER-/PR- (34.9%), ER+/PR- (9.3%) and ER-/PR+ (0%), [14]. In other study, the percentage of tumors expressing was ER+/PR+ 47%, ER+/PR- 12.2%,

ER-/PR+ 4.05% and ER-/PR- 36.8% (Ambroise, 2011). The variations in ER/PR status between our study and other studies may be due to thesample size, tumor grade and stage at time of presentation of the patients, and methods and techniques of evaluation, Blows *et al*. (Blows, 2010), in their analysis of data from 10,159 cases of breast cancer, concluded that differ subtypes of breast cancer (according to the expression of the markers), show distinct behaviors with differences in short term (<5years) and long term (>5years) prognosis and the worst prognosis in his study, at 15 year being the luminal HER2-positive breast cancer (Huang, 2005).

The present results found no significant correlation between expression of(ER+,PR+ and HER2) with age of patients. Other studies were showed a relationship between expression of ER and age in breast carcinoma (Huang, 2005). While Ayadi and coworkers (Ayadi, 2008), didn't find significant association between age and tumor expression of PR. In reference to the studied clinicopathological parameters, no association was found between HER-2immunopositivity and patient age although it has been claimed that an overexpression occur in younger patients. Younger breast cancer patients were reported to have higher frequency of HER-2but again no significant association with age (Panjwani, 2010). Other authors (Blows, 2010; Huang, 2005 and Seo, 2006), reported an association between HER-2 overexpression and younger age. This study revealed no significant correlation between ER+ / PR+ results with tumor largest diameter, histological grade, nodal involvement and pathological stage. Other study documented same results which revealed no association between hormonal receptors expression with (tumor largest diameter, lymph nodes and histological grade) (Ayadi, 2008).

Ivkovic'-Kapicl*et al*. (Ivkovic'-Kapicl, 2006), also observed that no significant relation between ER with nodal status and histological grade. Study from Iranby Pourzand*et al*. (Pourzand, 2011), revealed no significant association between the positive lymph nodes, tumor size and stage with ER and PR. Ambroise *et al.* (Ambroise, 2011) from Iran reported significant inverse association between (ER+, PR+) and tumor grade and lymph node status. Others recorded that ER and PR were inversely associated with histological grade (Pathak, 2011). Study by Nadji*et al*. (Nadji, 2005) of 6000 breast tumor noted that among infiltrating ductal carcinomas, most grade I tumors are ER-positive whereas only 2% of grade III carcinomas were ER positive. The differences in the rate of the results in different studies may berelated to the size of the sample, and to the difference in the tumor grade and stage and lymph node status of the studied groups. For example in our study, most of thecases (87.22%), are at grade II or III, and (93.62%) of our patients exceeded stage (I)at time of presentation.

This is in addition to the differences in laboratory techniques& processing. The current study revealed significant correlation between HER-2 expression and histological grade, but not with tumor size, or nodal status. This is closely comparable to the results of Ayadi *et al*. (Lakhtakia, 2015), and Selvarajan *et al*. (Selvarajan, 2006). Zhang *et a*l. (1915), and Sanft *et al*. (2015), were reported that the risk of recurrence for patients with hormone receptor–positive breast cancer may extend over a long period of time and about 50 % of recurrences are "late recurrence" (develop after the first 5 years of adjuvant antiestrogen therapy &follow-up).

**Conclusions**

Profiles for ER, PR IHC expression may be independent from the grade and stage of tumor. HER2 IHC expression associated significantly with tumor grade. Application of these markers in the clinical setting can explain the biological differences between the subtypes that may result in differences in response to specific therapies Further investigation is warranted to assess reproducibility of technique and investigate clinical implications of Breast Cancer Index (BCI) in primary breast carcinoma.

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