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ORIGINAL ARTICLE

Immunocytochemical Evaluation of Estrogen Receptor, Progesterone Receptor, and Human Epidermal Growth Factor Receptor 2 in Breast Cancer Cell Blocks and Corresponding Tissue Blocks: A Single Institutional Experience

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Background: Immunohistochemistry (IHC) is a routinely performed method to demonstrate estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) in surgical breast cancer specimens but not on cell block (CB) of fine-needle aspiration (FNA). The aims of this study were to evaluate the expression of ER, PR, and HER2 using immunocytochemistry (ICC) on CB and compare with the corresponding tissue blocks as gold standard as well as to compare with other similar studies. **Materials and Methods:** Forty-eight breast carcinoma CB specimens with their corresponding tissue blocks were identified. ICC on CB for ER, PR, and HER2 was performed and compared with tissue blocks. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value were measured for each receptor. The degree of agreement between CB and tissue blocks was calculated using Cohen's kappa (κ) test. **Results:** ER results showed 67.7% sensitivity, 94.1% specificity, 95.5% PPV, and a moderate agreement (κ =0.588). PR results showed 50% sensitivity, 90% specificity, 87.5% PPV, and a fair agreement (κ =0.368). HER2 results showed 58.3% sensitivity, 100% specificity, 100% PPV, and a moderate agreement (κ =0.539). **Conclusion:** The results of this study confirm the wide variations that occur between CB ICC and tissue block IHC in the detection of ER, PR, and HER2 in breast cancers. In comparison with other studies, we report a low sensitivity and high specificity rates for ER, PR, and HER2 in FNA CB. Further studies are recommended.

Key words: Breast cancer, cell block, estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, immunocytochemistry

INTRODUCTION

Evaluation of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression is routinely detected in routine formalin-fixed paraffin-embedded breast tissue blocks using immunohistochemistry (IHC).[1] However, IHC, being invasive, lengthy, and unsafe, has low acceptability to the deepest sites and expensive.[2] Fine-needle aspiration (FNA) is rapid, simple, and less expensive, and a cell block (CB) can be prepared from FNA sample materials.[3] This study aimed to evaluate the accuracy of ER, PR, and HER2 using immunocytochemistry (ICC) on CB and compared the results with the corresponding tissue blocks as gold standard as well as to compare with other similar studies.

MATERIALS AND METHODS

Case selection

FNA breast specimens were collected from the Department of Pathology from January 2007 to December 2016, over a period of 10 years. All FNA suspicious/malignant breast specimens were included in the study. Inadequate specimens,

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normal and benign cases, and cases without CBs and histopathological diagnosis were excluded from the study. Ethical approval was obtained from the local ethics committee.

Sample preparation

CB samples were initially fixed in a mixture of formalin, acetic acid, and alcohol for 1 h, followed by a fixation in 10% neutral-buffered formalin (10% NBF) for 6–24 h. CBs were first collected and then corresponded H and E slides. H and E slides were collected to evaluate the number of tumor cells. Any CB that did not have H and E slides, a new H and E slide was prepared. A 3 μm section was cut using a rotary microtome (RM2135, Leica Microsystems AG, Wetzlar, Germany) and then stained by H and E method.[4] Any H and E slides that contain less than ten tumor cells were excluded from the study. Slides that contain more than ten tumor cells were cut for ICC stain.

Immunocytochemistry on cell block

Slides were treated in PT-Link machine (Dako, Glostrup, Denmark), which includes deparaffinization, rehydration, and antigen retrieval for 1 h and then cooled for 15 min. Slides were then washed in Tris buffer for 5 min. Slides were incubated with 3% hydrogen peroxidase for 10 min. Slides were then washed again for 5 min. After that, slides were incubated with primary mouse monoclonal antibodies for ER at dilution 1:20 (6F11, Leica Biosystems Newcastle Ltd., UK), PR at dilution 1:200 (PgR636, Dako Cytomation, Carpinteria, CA, USA), and anti-HER2 ready to use (PATHWAY anti-HER-2/neu, clone 4B5, Rabbit Monoclonal Primary Antibody, Ventana Medical Systems, Arizona, USA) for 30 min at room temperature. Slides then were washed with Tris buffer three times each for 5 min followed with incubation for 30 min with secondary antibody (EnVision + System-HRP labelled polymer anti-Rabbit, Dako, Glostrup, Denmark). After that, the reaction was visualized using 3,3'-Diaminobenzidine (K3468, Dako, Glostrup, Denmark) for 2 min. Slides were counterstained with Mayer's hematoxylin for 2 min and then washed for 2 min in running tap water. Finally, slides were dehydrated, cleared, mounted in DPX, and examined by light microscope (Olympus, BX 51, Japan). Known positive and negative controls were run with each set.

Immunocytochemistry evaluation

ER and PR were graded using Allred scoring system which is based on the percentage of the cells that stained by ICC on a scale of 0–5 and intensity of the staining on scale of 0–3 giving a total score of 8. Nuclear staining ≥ 3 was considered positive.[5] HER2 was evaluated based on the membranous staining intensity 0–3. Tumor cells with 0 (absence) and 1+ (weak) were considered negative and 2+ (moderate) to 3+ (strong) were considered positive.[6] Slides were evaluated blindly by a pathologist.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 23 (SPSS Inc., Chicago, IL, USA). Accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value were then calculated using Chi-square test. The degree of agreement between CB and tissue blocks was calculated using Cohen's kappa (κ) test. Furthermore, *P* value of agreement significant was calculated. The *P* value of significance was hypothesized as no agreement between CB and tissue blocks scoring results. If the value is < 0.0005, the hypothesis is rejected. For κ test, perfect agreement was considered if the value was above 0.80. Good agreement was considered if the value between 0.61 and 0.80, and moderate agreement was considered for those ranged from 0.4 to 0.6. Values from 0.21 to 0.40 reflect fair agreement and < 0.2 attributed to poor agreement.

RESULTS

A total of 1041 FNA breast specimens were found. However, 993 cases were excluded as follows: 297 inadequate for cytological diagnosis, 80 normal breast tissues, 518 benign changes, 69 suspicious/malignant without CBs, 13 inadequate number of tumor cells in H and E slides prepared from CB sections, 11 unsatisfactory materials in CBs, 4 absent of histological results, and 1 case absent of CB. Only 48 malignant cases met with our criteria and those with corresponding tissue biopsies. The average age was 43.96 years, minimum of 27 years, and maximum of 78 years. Table 1 summarizes the types of tumors with their histological grades.

The results of ICC on CB and corresponding IHC on tissue blocks as the gold standard are shown in Table 2. ER showed 77.1% accuracy, 67.7% sensitivity, 94.1% specificity, 95.5% PPV, and a moderate agreement, κ =0.588. PR showed 66.7% accuracy, 50% sensitivity, 90% specificity, 87.5% PPV, and a poor agreement, κ =0.368. For HER2, accuracy, sensitivity, specificity, PPV, and κ-test were 76.2%, 58.3%, 100%, 100%, and 0.539, respectively. In comparison with other similar studies, the sensitivity of ER, PR, and HER2 is low, but the specificity is within the reported ranges [Table 3].

DISCUSSION

The evaluation of ER, PR, and HER2 in the diagnosis of breast cancer using CB is important for clinical management. Previous studies showed that the demonstration of these

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receptors on CB is possible.[3,7-11] The use of cytology direct smears, cytospin preparation, liquid-based preparation, and touch preparation with different fixatives to evaluate ER, PR, and HER2 in the diagnosis of breast cancer showed unsatisfactory results.[12,13]

In the literature, the sensitivity ranges for ER, PR and HER2 were 82%–98% , 56%–92% and 70%–100% and for specificity 70%–100%, 70%–100% and 81%–100%, respectively.[3,7-10] In the current study, the sensitivity of ER, PR, and HER2 was 67.6%, 50%, and 58.3% and specificity was 94.1%, 90%, and 100%, respectively. Those findings show that the sensitivity is relatively low when compared with other studies, whereas the specificity is well within the reported ranges.[3,7-10] Several factors can affect the sensitivity of ICC method on CB, and these include duration and type of fixative, antigen retrieval method, type of primary antibody, quality of CB sections, and possibly the interpretation of those receptors.

Table 1: Types of breast cancer tumors with their histological grades

Types	*n*	Histological grade
Invasive ductal carcinoma	29	Grade 2=11 Grade 3=18
Infiltrating ductal carcinoma	7	Grade 2=2 Grade 3=5
Papillary ductal carcinoma	1	No histological grade
Invasive lobular carcinoma	3	Grade 1=1 Grade 2=2
Invasive ductal carcinoma with lobular features	1	No histological grade
Invasive micropapillary ductal carcinoma	2	Grade 3
Invasive ductal carcinoma with apocrine	2	Grade 2=1 Grade 3=1
Invasive ductal carcinoma with mucinous	2	Grade 2
Invasive ductal carcinoma with medullary like	1	Grade 3
Total	48	

Fixation is the most important step in IHC and ICC. In this study, a mixture of formalin, acetic acid, and alcohol was used first to promote cellular aggregation followed by 10% NBF. Prolonged formalin exposure to cells might mask ER, PR, and HER2. It was reported that prolonged formalin fixation may result in a weak staining.[14] Most of the previous studies use 10% NBF and keep for 6–24 h.[3,7-10] Other studies used first 95% alcohol followed by 10% NBF and found that the expression of ER, PR, and HER2 was not affected.[3] However, another study reported that ethanol-fixed CBs showed an increased false-positive HER2 expression in breast cancer cases.[15] According to the American Society of Clinical Oncology/College of American Pathologist (ASCO/CAP), breast specimens should be fixed from 6 to 48 h.[6,16]

Antigen retrieval is another important step in IHC and ICC. In the current study, PT-linked machine on citrate buffer at pH 9.5 was used for both methods, IHC and ICC. Other study used pressure cooker on citrate buffer at pH 6 and reported that sensitivity in the diagnosis of breast cancer cases was 94%, 70%, and 70.6% for ER, PR, and HER2, respectively.[10] It is important to note that the same antigen retrieval method used for ER, PR, and HER2 in tissue blocks.

Currently, there are three in use clones for ER mouse monoclonal antibodies (SP1, 6F11, and 1D5).[17] In the current study, 6F11 clone at a dilution 1:20 was used. Other studies reported that SP1 and 6F11 antibodies were more sensitive than 1D5 in the detection of ER.[17] Regarding PR, there are two in use antibody clones, PgR636 and PgR1294, and both showed similar findings.[18] In the current study, PgR636 at a dilution of 1:200 was used to detect PR in the breast cancer cases. Regarding HER2, there are seven different antibodies tested: one RabMab (SP3, NeoMarkers), two rabbit polyclonal antibodies (A0485, DAKO), three mouse monoclonal

Table 2: Correlation of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 status between tissue blocks and cell block in the diagnosis of breast carcinomas

CB		Tissue blocks		Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cohen's kappa test	*P*
		+	-							
ER	+	21	1	77.1	67.7	94.1	95.5	61.5	0.588	<0.0005
	-	10	16							
PR	+	14	2	66.7	50	90	87.5	56.2	0.368	<0.0005
	-	14	18							
HER2	+	14	0	76.2	58.3	100	100	70.6	0.539	0.000
	-	10	24							

CB=Cell block; ER=Estrogen receptor; PR=Progesterone receptor; HER2=Human epidermal growth factor receptor 2; NPV=Negative predictive value; PPV=Positive predictive value; +=Positive; -=Negative

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Table 3: Comparison of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 findings of the current study with other similar studies

	Bueno Angela *et al*., 2013[3]	Hanley *et al*., 2009[7]	Shabaik *et al*., 2011[8]	Vohra *et al*., 2016[9]	Kinsella *et al*., 2013[10]	Current study
Receptors						
Number of samples	62	41	39	131	50	48
ER						
Sensitivity (%)	92.7	87.1	85.7	98.9	94	67.7
Specificity (%)	85.7	100	100	87.8	89	94.1
PR						
Sensitivity (%)	92.7	56.3	80	83.3	83.3	50
Specificity (%)	97.7	92	100	70.2	70	90
HER2						
Sensitivity (%)	70	95.4	100	100	70.6	58.3
Specificity (%)	100	68.4	100	100	81.8	100

ER=Estrogen receptor; PR=Progesterone receptor; HER2=Human epidermal growth factor receptor 2

antibodies (NCL-CB11, Novocastra; CM-CB11, Cell Marque; 4D5, Genentech), and one rabbit monoclonal antibody (4B5, Ventana).[19] The current study used anti-HER2 clone 4B5. A study reported that 4B5 is a sensitive marker for HER2 expression in gastric cancer cases.[20] Above used antibody clones in the current study are also used to demonstrate ER, PR, and HER2 in tissue blocks.

In this study, the interpretation of ER, PR, and HER2 was per the ASCO/CAP recommendations.[6] However, for ER and PR interpretation, confusion might occur between scores 2, which is negative and 3, which is positive when using Allred scoring system.[10] ICC on CB for ER showed four cases to have 2 as a total score in Allred system. Three of those four cases showed a total score of 5 and above in IHC in tissue blocks. For PR, ICC on CB showed seven cases to have 2 as a total score. Five of those cases were above 3 in IHC in tissue blocks. For HER2, confusion might occur between + 1, which is negative, and + 2, which is positive. ICC on CB showed only one case of + 1, which turned to be + 2 in IHC in tissue blocks.

The quality of CB sections could be related to the interpretation of ER, PR, and HER2. This study dealt with CBs from 2007 to 2016. Trimming and sectioning of those blocks might lose or reduce the number of tumor cells, even that we initially excluded blocks that contained no tumor cells. Other less important factors such as bloody samples, paucicellular specimens, and sampling problems may alter the expression of ER, PR, and HER2 in CB.[7]

Despite the low sensitivity of ER and HER2, a moderate positive agreement, weighted κ of 0.588 and 0.539, was obtained between CB and tissue blocks, respectively. We found that invasive ductal carcinoma was the most common type of breast cancer with 62.5%. This finding is in agreement with other studies.[15,21] Furthermore, we noticed that Grade G3 was the most frequent histologic grade with 56.25%, followed by Grade G2 with 37.5%. Other study revealed that G2 was the most frequent histologic grade followed by G3 with 47.5% and 30.3%, respectively.[21] As a limitation of this study, we should point out that cases with neoadjuvant or adjuvant chemotherapy were not excluded from the study. Furthermore, this study was retrospective on tissue blocks and prospective on CB.

CONCLUSION

The results of this study confirm the wide variations that occur between CB ICC and tissue block IHC in the detection of ER, PR, and HER2 in breast cancers. In comparison with other studies, we report low sensitivity and high specificity rates for ER, PR, and HER2 in FNA CB. Further studies are recommended.

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Conflicts of interest

There are no conflicts of interest.

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