EVALUATION OF HER-2/NEU EXPRESSION IN CELL BLOCK PREPARATION IN BREAST CARCINOMA IN BANGLADESHI WOMAN

Md Saiful Islam[1*] Mehnaz Nasreen[2] Sayeeda Nasreen[3] M Shahab Uddin Ahamad[4]
Pradip Bhattacharjee[4] Md Zillur Rahman[4]

Abstract

Background: HER-2 overexpression is associated with clinical outcomes in patient with breast carcinoma. Particularly this is important for target therapies with the monoclonal antibody (Trastuzumab). With aspirated materials from breast carcinoma more information may be retrieved by making cell blocks like immunohistochemistry in the inoperable breast cancer patient. **Materials and methods:** This study was carried out on 102 female patients with palpable breast lump, among them 33 malignant cases were found and cell blocks were made from the aspirated materials. HER-2 statuses were estimated in both the cell block preparation and histopathological blocks separately. **Results:** Among the 33 cases of duct cell carcinoma, cell blocks of 32 cases were found to be adequate but one of them died before the surgery. HER-2 expression score of 0, 1+, 2+ and 3+ were foundin 14, 7, 5 and 5 cases respectively. All the 14 cell block scored as '0' also showed similar '0' score in their corresponding histologic block. Among the seven cell blocks scored as '1+', 5 cases showed '1+' staining and 2 case showed '2+' staining in their corresponding histologic blocks. Of the '2+' cases in cell block, 2 cases failed to correspond as they scored '1+'. Finally 5 cell blocks with '3+' scorecorresponded with that of the histologic blocks.In cell block immunohistochemistry, the sensitivity was 71.43%, specificity 91.67%,positive predictive value of 71.43% and a negative predictive value of 91.67% with an accuracy rate of 87.71% with a highly significant p value (<.001). Cohen's Kappa test of agreement was 0.814 which means there was a good agreement and Spearman's rank-correlation analysis found to 0.984 with a p value of < .001 which was highly significant. **Conclusion:** This study found overexpression of HER-2 can be determined with confidence in the cell block preparation, particularly in the negative cases.

Key words
Breast carcinoma; Cell block; Immunohistochemistry; HER-2/neu.

Introduction

In the management of invasive breast cancer three molecular markers are used in the routine clinical management: Estrogen Receptor(ER) Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor -2 (HER-2) or frequently called HER-2/neu as it was derived from a rodentglioblastoma cell line, a type of neural tumor. All are targets and/or indicators of highly effective therapies against invasive breast cancer in various clinical settings[1]. Breast cancer is the first type of solid cancer to be successfully treated with molecular targeting therapy, the target is being HER-2/neu[2]. In 2013 The Update Committee of American Society of Clinical Oncology-College of American Pathologist (ASCO-CAP) recommended that HER2 status (HER2 negative or positive) should be determined in all patients with invasive (Early stage or recurrence) breast cancer on the basis of one or more HER2 test

1. Assistant Professor of Pathology
Rangamati Medical College, Rangamati.
2. Associate Professor of Pathology
Rangamati Medical College, Rangamati.
3. Assistant Professor of Pathology
Chittagong Medical College, Chittagong.
4. Associate Professor of Pathology
Chittagong Medical College, Chittagong.

***Correspondence: Dr. Md Saiful Islam**
Email: biddut136@gmail.com
Cell : 01715 254573

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results (Negative, equivocal, or positive). They recommended a must request HER2 testing on every primary invasive breast cancer (And on metastatic site, if stage IV and if specimen available) from a patient with breast cancer to guide decision to pursue HER2-targeted therapy[3]. Immunohistochemical (IHC) stains for breast carcinoma biomarkers are currently performed on a patient's biopsy or surgical resection. The use of cytologic samples for determining a patient's Estrogen Receptor (ER) Progesterone Receptor (PR) and Human Epidermal Growth Factor Receptor-2 (HER-2) statuses has yet to be validated[4]. In practice, immunohistochemistry using cell blocks, and also core biopsies, for the assessment of HER-2, ER and PR is mainly needed for inoperable tumors and recurrences. However, assessment of HER-2 is important pre-operatively to predict the response of the tumor to neoadjuvant chemotherapy and trastuzumab, allowing diminution of tumor size for a less aggressive surgical procedure[5].

The aim of the study was to assess the reliability of immunohistochemistry for HER-2 in cell block preparations of breast cancer patients.

Materials and Methods

The study was conducted from January 2012 to December 2012 in the Department of Pathology, Chittagong Medical College, Chittagong, Bangladesh. Female patient presented with clinically diagnosed breast lump were enrolled except those were diagnosed and on chemo or radiotherapy. Fine Needle Aspiration (FNA) was done to make smears and cell block. Following smear preparations, fine needle aspirated materials from the needles and syringes were rinsed in 10 ml of 10% formalin in a test tube. The entire material was centrifuged in a 10-mL centrifuge tube to create a cell pellet. The supernatant fluid was decanted and the deposit fixed in freshly prepared 10% formalin. Cell block sample was allowed to fix initially for 4-12 hours. Then the cell block was processed, sectioned and stained with hematoxylin and eosin and later on for HER-2 immunohistochemistry. Later malignant breast tumor underwent surgery, and then histopathological diagnosis of biopsy specimen was done subsequently. HER-2 status was examined in both the histological and cell block sections and results were recorded.HER-2 results were determined according to the ASCO/CAP guidelines[6]. Cell blocks containing at least 100 cells per cell block were included in the study for Immunohistochemistry (IHC). Statistical analysis was done by Statistical Package for Social Sciences (SPSS) version 17. Immuno-staining results for HER-2 on cell blocks and on tissue sections were analyzed for determining sensitivity, specificity, Positive Predictive Value (PPV) Negative Predictive Value (NPV) and accuracy. Spearman rank correlation analysis (ρ) and Cohen kappa (κ) test of agreement were done. Kappa values above 0.6 correlated with good agreement, 0.4-0.6 was considered moderate agreement, whereas kappa results below 0.4 corresponded to fair, below 0.2 reflected poor agreements.The studywas approved by the Ethical Committee of Chittagong Medical College, Chittagong.

Results

Table I : Results of HER-2 in cell blocks and corresponding tissue blocks

DCC Sl. No.	Cell Block HER-2	Tissue Block HER-2
01	0	0
02	0	0
03	0	0
04	2+	1+
05	0	0
06	0	0
07	Inadequate	3+
08	0	0
09	1+	2+
10	0	0
11	3+	3+
12	0	0
13	0	0
14	2+	1+
15	0	0
16	3+	3+
17	Died	
18	1+	1+
19	1+	1+

DCC Sl. No.	Cell Block HER-2	Tissue Block HER-2
20	0	0
21	0	0
22	0	0
23	0	0
24	1+	1+
25	3+	3+
26	2+	2+
27	1+	1+
28	2+	2+
29	1+	2+
30	3+	3+
31	2+	2+
32	1+	1+
33	3+	3+

Table II : Distribution of HER-2 status on cell blocks and corresponding tissue blocks (n = 31)

HER-2 Status ●	Cell Block n (%)	Tissue Block n (%)
Positive	5 (16.1)	5 (16.1)
Equivocal	5 (16.1)	5 (16.1)
Negative	21(67.8)	21 (67.8)

● 3+ ~ Positive, 2+ ~ Equivocal, 1+ 0 ~ Negative

Table III : Statistical values of HER-2 expression in cell blocks and tissue blocks (n = 31)

Validity test	Results
Sensitivity	71.43%
Specificity	91.67%
Positive predictive value	71.43%
Negative predictive value	91.67%
Accuracy	87.1%

Table IV : Correlation between HER-2 expression in cell blocks and tissue blocks (n = 31)

Correlation tests	Results
Cohen's Kappa κ (p)	0.814 (< 0.001)
Spearman's Correlation ρ (p)	0.967 (< 0.001)

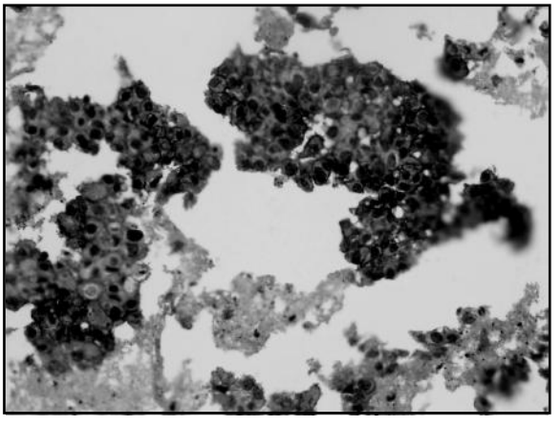

Fig 1 : HER-2 expression '3+' in cell block

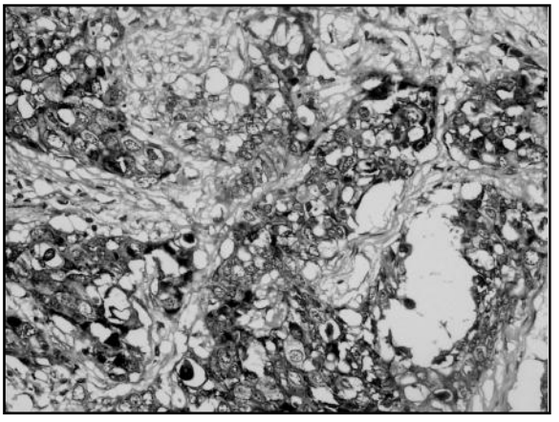

Fig 2 : HER-2 expression '3+' in tissue block

Among the 33 cases of duct cell carcinoma, 32 cell blocks were found to be adequate and one cell block specimen was inadequate. Of the 32 cases, one patient died before the operation, so no histologic block was available. All 32 cell blocks and histologic blocks were stained form HER- 2 expressions (Table I). HER-2 expression score in 14 cell blocks were '0', 7 cases were scored as '1+', 5 cases showed '2+' staining and 5 cases showed '3+' staining. All the 14 cell block scored as '0' also showed similar '0' score in their corresponding histologic block. Among the 7 cell blocks scored as '1+', 5cases showed '1+' staining and 2 case showed '2+' staining in their corresponding histologic blocks. Of the '2+' cases, scored in cell block, 3 case showed similar 2+ staining in the histologic block and failed to correspond with their histologic blocks in other 2 cases as they scored '1+'. Finally 5 cell blocks with '3+' staining were also '3+' in their corresponding histologic blocks (Figure 1 & 2).

Taking histological block as the gold standard, of the 31 cases 5 was positive (3+), 5 was equivocal (2+) and 21 (1+ or 0) were negatives. Taking the score of 3+ and 2+ as the criterion of positivitythis study found finally there were 5 true positives, 22 true negatives, 2 false negatives and 2 false positive cases when compared with histologic blocks (Table II)[5,7].

In cell block immunohistochemistry, the sensitivity was 71.43%, specificity. 91.67%, positive predictive value of 71.43% and a negative predictive value of 91.67%with an accuracy rate of 87.1% (Table III). Cohen's Kappa test of agreement and Spearman's rank-correlation analysis were found to be 0.814 and 0.967 respectively (Table IV).

Discussion

The aim of this study was to find out the reliability of immunohistochemistry for HER-2 in cell blocks like that of histologic block. The total number of patient was 102, among them 33 were malignant and subjected for immun histochemistry. Considering histologic block as gold standard, this study found 6(18.2%) cases with3+ staining for HER-2 in the histologic blocks. This finding is consistent with American Society of Clinical Oncology and the College of American Pathologists ((ASCO/CAP) guidelines which estimated that the human epidermal growth factor receptor- 2 (HER-2) gene is amplified and/or over expressed in approximately15% to 20% of primary breast cancers[6]. Previously in this country Hossain et alfoundHER-2 over expression in 38.4 % (96/250) cases and Mostafa et alin 28.4% (95/335) cases[8,9]. In IndiaShirsat et alfound 25.58% positive cases[10]. Their findings were much higher and not concordances with the guidelines of the ASCO/CAP[6]. The reasons of the difference from their findings may be due to larger sample size of their study. Though there are many factors believed to contribute in the result of overexpression of HER-2, like fixation time, antibody storage and concentration, interobserver variation. However Angela et aland Shin et alreported 16.1% and 20% positive cases which are comparable to the findings of the present study[5,11].

In this study, out of 31 cell blocks, HER-2 was positive in 5/31 cell blocks and negative in 26/31 cell blocks (5 were 2+, 6 were 1+ and 14 were 0) along with one cell block having inadequate materials. Comparing with the findings of HER-2 expression in histologic blocks there were 4 discrepant case, two case with 1+ in cell block showed 2+ in histological block and two cases of 2+ in cell block showed 1+ in histologic block. One cell block sample was inadequate and the cause may be the size of that tumor (> 5 cm) and aspiration brought out mostly necrotic material. So finally there were five true positives, twenty two true negatives, two false negatives and two false positive giving a 71.43% sensitivity and 91.67% specificity. Those yielded a positive predictive value of 71.43% and a negative predictive value of 91.67% with an accuracy rate of 87.09%. Angela et alreported sensitivity of 70.0%, specificity and PPV of 100.0%, NPV of 94.5% and accuracy of 95.2% in their study[5]. Except the positive predictive value, all other indicators are nearly similar to findings of this study. Taking histology as the final outcome Kumar et al showed the sensitivity for demonstration of HER2 on cell block 91.7% and specificity was 81.5%[12]. Shabaik et alshowed HER-2 by immunohistochemistry on both cell block and tissue performed in 40 cases and there was 100% sensitivity and specificity ($p < 0.001$)[7]. This study found good agreement (κ, 0.814) with a 'p' value of <0.001 considering HER-2 expression in cell block and histologic block. And the Spearman's rank correlation (ρ) showed a value of 0.967 with a 'p' value of <0. 001, whichwas highly significant. Hanely et al showed good positive agreement (87.5%) and fair overall agreement (ρ, 0.45) between Needle Core (NC) and Cell Block (CB) preparations when evaluating HER-2 positive cases (3+) and HER2-negative cases[13]. Williams et alshowed fifteen of thirty-four total cases (44.1%) had HER-2/neu IHC discrepant results between FNA ethanol-fixed CB and formalin-fixed Tissue Block (TB) samples. Of those 15 cases, 7 (46.7%) had either equivocal HER2/neu staining (4 cases) or positive HER-2/neu staining (3 cases) by ethanol-fixed cell block IHC but were negative by formalin-fixed TB IHC. A moderate positive agreement of 73.3% (Weighted Kappa of 0.571) was obtained between ethanol-fixed and formalin-fixed tissues with HER-2immunohistochemistry with a Spearman's correlation of 0.56 (<0.002) by them[14]. Hanley et

al and Williams et al in their studies concluded that HER-2 testing on CB is not reliable because results did not correlate with tissue IHC, however, in their studies the cell blocks were fixed in 50% ethanol[13, 14]. The American Society of Clinical Oncology / College of American Pathologists Guidelines recommend using only formalin fixation for HER-2 testing samples[3].

Shabaik et alconcluded that though occasionally cell blocks may be acellular or specimens are not sufficient to prepare cell blocks, 100% correlation for both positive and negative HER-2 testing results by immunocytochemical analysis can be determined by using the recommended ASCO/ CAP scoring guidelines[7]. Tumors that have equivocal (2+) staining for HER-2 have poor interobserver reproducibility and should be evaluated further by Fluorescent In Situ Hybridization (FISH) for HER-2 gene amplification, because 8% to 25% or even up to 48% of tumors can reveal HER-2 amplification with this method[3]. It is well known that several factors, including preanalytic factors (Length and time of tissue fixation) analytic factors (Type of antibody, antigen retrieval) and post-analytic factors (Interpretation of results) can have major implications on the accuracy of results. Inter-laboratory and intra-laboratory variability can be reduced by strict adherence to the national guidelines established by ASCO/CAP. In addition, the presence of an internal control is helpful if there is concern about false-negative results. However, internal control (Non-neoplastic) breast tissue usually is absent in cytology preparations. Overall, there are many confounding factors (Bloody sample, paucicellular specimen, use of alternative fixative, sampling problems) that can alter antigen expression/detection and, thus, can influence IHC results on cytologic preparations. Brifford et alalso mentioned the risk of sample contamination by ductal carcinoma in situ as a major problem regarding use of cytological material for immunohistochemical study[15].

The major limitation of this study is its smaller number of cases. It was due to time and resource constrain. Besides, all the patients were included from a single centre of Bangladesh, so it may not reflect the exact scenario of this country. Another issue is that there was no scope for doing FISH in equivocal cases which is very critical. This study was limited only to determine HER-2 status but should have the aim to test estrogen receptor and progesterone receptor status.

The major finding of the study is that in positive (3+) and negative (0) HER-2 cases there are 100% correlation between cell block and histologic preparation. In equivocal cases the various factors described earlier may play the roles for different findings between cell block and histologic block and FISH should have been done in these cases.

A further study with larger size of sample and FISH for HER-2 along with ER and PR status needs to be carried out as the results may produce beneficial results for the patients with inoperable breast carcinoma.

Conclusion

From this study we can make inference that HER-2 expression can be seen in the cell block preparation from the aspirates of malignant breast lump.HER-2 expression in the cell block preparation would help the inoperable cases of malignant breast lump to initiate neoadjuvant chemotherapy.

Disclosure

All the authors declared no competing interest.

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