RESEARCH COMMUNICATION

Determination of HER-2/neu by Chromogenic in Situ Hybridization on Borderline (2+) Immunohistochemistry Cases in Carcinoma Breast

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Abstract

Objective: To determine the HER-2/neu status by chromogenic in situ hybridization (CISH) on tissue specimens with a borderline (2+) immunohistochemistry (IHC) score in carcinoma breast by a descriptive, cross-sectional study in the Histopathology Department, Armed Forces Institute of Pathology (AFIP), Rawalpindi from Jun 2008 to Dec 2009. Methods: Tissue block specimens from 50 consecutive patients having HER-2/neu score of borderline (2+) on IHC assay were tested for HER-2/neu gene amplification by CISH. Mean and standard deviation were calculated for quantitative variables like age and HER-2/neu gene copy signal/clusters by using SPSS version 14. Frequencies and percentages were also calculated for qualitative variables like type of carcinoma and results of HER-2/neu by CISH (amplified/nonamplified). Results: HER-2/neu gene amplification by CISH was found in 10 (20%) out of 50 patients with borderline (2+) IHC score. All CISH amplified cases belonged to invasive ductal carcinoma type. No significant correlation was noted between type of carcinoma and HER-2/neu gene amplification. Conclusion: Chromogenic in situ hybridization (CISH) is a practical, cost-effective and reliable method for analysis of HER-2/neu borderline (2+) cases which may be candidates for Herceptin therapy.

Keywords: Breast carcinoma - chromogenic in situ hybridization - HER-2/neu - immunohistochemistry

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Introduction

Breast carcinoma is the most common malignant tumor and leading cause of cancer death in women, with more than 1,000,000 cases occurring worldwide annually (Rosai, 2004). According to the survey of epidemiology and end results (SEER) data, the world wide incidence of breast carcinoma is 123 women per 100,000 and its incidence in Asia is 89.5 per 100,000 (Horner et al., 2009). The breast carcinoma is a most prevalent cancer in Pakistan with approximate prevalence of 14.2% (Khan et al., 2004). Prognosis and management of breast carcinoma is influenced by classical variable such as tumour histologic type and grade, tumor size, proliferation index, lymph node status, lymphatic / blood vessel invasion, status of hormone receptors - estrogen receptor (ER) and progesterone receptors (PR) of the tumour and more recently HER-2/neu status (Sinczak-Kuta et al., 2007).

The human epidermal growth factor receptor 2, HER-2 also known as HER-2/neu or c-erbB-2 is an oncprotein located on chromosome 17q21. It is overexpressed in 20% to 30% of invasive breast carcinomas and is associated with the risk of aggressive disease and poor patient survival (Bundred, 2001; Vera-Roman and Rubio-Martinez, 2004).

Several studies have supported the importance of HER-2/neu overexpression as an independent prognostic marker of clinical outcome. Patients with breast tumors overexpressing HER-2/neu benefit from doxorubicin based chemotherapeutic regimen. It is also useful as a treatment predictor for the monoclonal antibody trastuzumab (Herceptin) (Madrid and Lo, 2004).

Therefore, determination of HER-2/neu status is an integral part of clinicopathological workup in patients with breast carcinoma (Isola et al., 2004). The HER-2/neu status can be analyzed by immunohistochemistry (IHC) or in situ hybridization (ISH) techniques like fluorescent in situ hybridization (FISH) and more recently, Chromogenic in situ hybridization (CISH) on formalin fixed paraffin embedded tissue sections. IHC is simple, relatively inexpensive, and can be performed quickly with little technical difficulty (Elkin et al., 2004). Studies done in the west have shown false/weak positive results for HER-2/neu overexpression on IHC assays owing to the variation in specimen processing, reagents and staining interpretation. Interlaboratory
variation in results found during studies also resulted in wrong selection of patients for transtuzumab therapy subsequently adding extra financial burden to the patients (Gupta et al., 2003; Hanna and Kewok, 2006).

CISH testing is sensitive and specific in detecting HER-2/\textit{neu} gene amplification. It is also practical, cost effective and convenient to perform as it does not require special equipment like fluorescent microscope and much experience in interpreting results (Vocaturo et al., 2006). Direct evaluation of gene amplification using CISH assay is a reliable method for routine diagnostic evaluation of HER-2/\textit{neu} status in breast cancer patients, especially clinical specimens showing 2+ IHC score (Di Palma et al., 2007).

There is no local study available on this subject. The rationale of our study is to determine HER-2/\textit{neu} status by CISH assay on patients with borderline (2+) IHC score for HER-2/\textit{neu} in our population. The results of this study should prove helpful for the clinicians/Oncologists to select the right patients for Herceptin (transtuzumab) therapy.

Materials and Methods

It was a descriptive study at a tertiary care diagnostic laboratory. A total of fifty consecutive patients diagnosed as having HER-2/\textit{neu} borderline (2+) immunohistochemistry score from 16 June 2008 to 31 December 2009, Armed Forces Institute of Pathology (AFIP), Rawalpindi were included in the study. Immunohistochemistry assay for HER-2/\textit{neu} was performed using Novacstra’s kit as per manufacturer guidelines.

All the 2+ cases were subjected to CISH assay by Zymed laboratories. The results of CISH assay were interpreted on light microscope. More than 10 individual signals or clusters in more than 50% of tumour cells were considered as high amplification and signals between 5 and 10 were considered as low amplification. Signals less than 5 were considered non amplified. Histological type of carcinoma was also noted.

Mean and standard deviation were calculated for quantitative variables like age and HER-2/\textit{neu} gene copy signal/clusters by using SPSS version 14. Frequencies and percentages were also calculated for qualitative variables like type of carcinoma and results of HER-2/\textit{neu} by CISH (high amplified/low amplified/nonamplified).

Results

A total of 50 patients of breast carcinoma with immunohistochemical score of HER-2/\textit{neu} 2+ (irrespective of patient’s age, sex, parity and marital status) were evaluated in the study. The mean age of the patients was 59.8 ± 14.6 (Mean ± SD) years. Their ages ranged from 23 to 97 years with a median age of 64.5 ± 14.6 years. Most of the patients (n=21, 42.0%) were above the age of 40 years. Majority of the patients were in fifth decade followed by fourth and seventh decades.

Out of the 50 patients, 48 (96.0%) were females and 2 (4.0%) were males with a female to male ratio of 24:1. Histologically, 46 (92.0%) patients had invasive ducal carcinoma, 3 (6.0%) had invasive lobular carcinoma and 1 (2.0%) had mucinous carcinoma. The HER-2/\textit{neu} gene amplifications by CISH were seen in 10 (20%) patients and 40 (80%) cases were interpreted as non-amplified. All the CISH amplified cases belonged to invasive ductal carcinoma group and all patients were females.

Among the CISH amplified cases, 4 (8%) had high level of gene amplification by scoring more than 10 signals and clusters (Figure 1). Six cases (12%) were scored as having average 7 signal indicating low gene amplification (Figure 2). Among the CISH non amplified cases, 33 (66%) cases showed average signal numbers 2 to 5 whereas 7 (14%) cases had signal numbers 0 to 2 (Table 1).

<table>
<thead>
<tr>
<th>CISH gene signals/clusters</th>
<th>No. of cases</th>
<th>%</th>
<th>Amplified/non amplified</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10 signals and scattered clusters</td>
<td>4</td>
<td>8%</td>
<td>Amplified</td>
</tr>
<tr>
<td>Average 7 signals</td>
<td>6</td>
<td>12%</td>
<td>Amplified</td>
</tr>
<tr>
<td>2-5 signals</td>
<td>33</td>
<td>66%</td>
<td>Non amplified</td>
</tr>
<tr>
<td>0-2 signals</td>
<td>7</td>
<td>14%</td>
<td>Non amplified</td>
</tr>
<tr>
<td>50</td>
<td>100%</td>
<td></td>
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Table 2. Frequency of HER-2/\textit{neu} Gene Amplification by CISH on 2+ IHC Cases in Different Studies

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>No. of 2+ amplified cases</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>46.4%</td>
<td>13 (n=28)</td>
<td>Zhang et al., (2006)</td>
</tr>
<tr>
<td>35.5%</td>
<td>6 (n=17)</td>
<td>Peiro et al., (2007)</td>
</tr>
<tr>
<td>69.2%</td>
<td>9 (n=13)</td>
<td>Hauser et al., (2004)</td>
</tr>
<tr>
<td>27.0%</td>
<td>20 (n=75)</td>
<td>Van de Vijver et al., (2007)</td>
</tr>
<tr>
<td>53.0%</td>
<td>19 (n=36)</td>
<td>Amina et al., (2006)</td>
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Discussion

Chromogenic in situ hybridization (CISH) is a recent methodology, which was introduced as an alternative to Fluorescent in situ hybridization (FISH). In this method, HER-2/neu gene copies are detected using a permanent peroxidase reaction and the results can be visualized by light microscopy, which makes it easy to observe both the tissue morphology and the gene amplification.

Over expression and/or amplification of the HER-2/neu gene, represents a new risk category in breast carcinoma, especially in the algorithm for the selection of adjuvant systemic treatments. Moreover, the widely documented link between HER-2/neu status and response to endocrine and anthraccline based therapies, as well as the recent trials for trastuzumab efficacy in the adjuvant and neoadjuvant settings, makes it mandatory a standardized, accurate, and reproducible determination of HER-2/neu status on histological specimens. FISH is in generally considered as the gold standard method for detecting HER-2/neu gene amplification. The recent introduction of CISH assay provides an attractive alternative to FISH. CISH is advantageous, it allows simultaneous evaluation of gene copy numbers, tumor cells, and detailed surrounding tissue morphology on the same histological slide (Isola et al., 2004; Bhargava et al., 2005).

Usually, HER2/neu testing algorithm starts with immunohistochemical analysis. Whether a patient is eligible for anti-HER-2 therapy depends on clear-cut HER2/neu status determined by immunohistochemical analysis. Cases with equivocal (2+) immunohistochemical results should be restested and confirmed with CISH/FISH. Because of the technical difficulties, expensive equipment requirements in FISH, Chromogenic in situ hybridization (CISH) has been evaluated as a potential alternative. Like FISH, it measures the degree of HER-2/neu gene amplification but is more straightforward than FISH on scoring (Arnould et al., 2003).

In the recent days, CISH has been gaining in popularity, mainly because of the simplicity of the procedure and ease of signal scoring as compared with FISH. It has also been approved by FDA. The development of newer equipment and the availability of an autostainer, it expedites the counterstain steps and has led to the wide acceptance of CISH. A number of studies have evaluated the reliability of CISH, these were mostly based on the experience of a single institution with a relatively small number of cases. The reported overall agreement between CISH and FISH was 84% to 100% (Arnould et al., 2003).

Keeping in view the significance of resteting HER-/neu 2+ cases by CISH, the present study was designed with an aim to evaluate gene amplification in these borderline cases as seen on IHC. These patients may be the potential candidate for anti-HER-2/neu monoclonal antibody (Transtuzumab, Herceptin).

Results of frequency of HER-2/neu gene amplification by chromogenic in situ hybridization on 2+ immunohistochemistry cases from different studies are shown in Table 2. The results were as high as 69.2% in a study by Hauser and Dandachi (2004), where as in a study by Van de Vijver et al18, Her-2/neu gene amplification was detected in 2+ cases in 27% cases. The results from other studies also show variable percentage of results. In our study, the results were 20%. It was lowest among all the studies done worldwide. The reasons behind these results may the differences in tissue handling, fixation, interobserver and interlaboratory variability in interpretation of HER-2/neu 2+ cases by pathologist and CISH staining protocols. Moreover, this study is the first ever done in this part of the world.

Among the type of carcinoma, all the CISH amplified cases belonged to invasive ductal carcinoma in our study. No significant correlation was seen between the histological type of breast carcinoma and HER-/neu gene amplification by CISH. These results were comparable with an international study (Madrid and Lo, 2004).

In conclusion, based on the present results, which need to be confirmed by a larger study, we suggest that CISH can be considered a useful, simple, and reproducible method for detecting HER-2/neu gene amplification in cases with borderline (2+) immunohistochemistry score. Such patients should get benefit from Herceptin (Transtuzumab) therapy.

References


