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Investigation of Equivocal Expression of HER2 Gene by Immunohistochemistry in Patients with Invasive Breast Ductal Cancer Confirmed by CISH or FISH and Its Relationship with Clinicopathologic Variables

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Abstract

Objectives: The aim of this study was to determine the equivocal HER2 gene expression in patients with invasive ductal carcinoma of the breast approved by the chromogenic in situ hybridization (CISH) or fluorescence in situ hybridization (FISH) method and its association with clinicopathologic variables.

Methods: This cross-sectional census-based study was conducted with information obtained by filling out questionnaires based on patient records or telephone contacts. Referring to the pathology lab archive, we extracted all immunohistochemistry (IHC) results related to the patients of breast cancer with HER2 2+ between 2011 and 2016. The patients' information and the results of the CISH or FISH method were obtained, as well.

Results: The mean age of 247 patients was 48.21 $\pm$ 11.33 years. The result of the complementary CISH or FISH test was negative in 124 (50.2%) patients and positive in 123 (49.8%) patients. There was a significant relationship between the age groups and CISH (P value $<$ 0.05). In the age group of fewer than 29 years, there was the most likelihood of CISH positivity and in the age group of 60, there was the most likelihood of CISH negativity. There was a significant relationship between KI-67 status and CISH result. Our results revealed that false negative HER2 test was high.

Conclusions: this result may be due to the type of the kit or antibody used or different guidelines used for the interpretation of the test. It is proposed to perform more precise complementary tests to determine the status of this gene. Moreover, laboratories should follow the ASCO/CAP guideline for HER2 evaluation in breast carcinoma.

Keywords: HER2 Gene, Immunohistochemistry, Breast Ductal Cancer

1. Background

Breast cancer is one of the most common cancers and one of the main causes of death among women (1). The course of breast cancer is affected by several predicting factors including tumor grade, tumor size, cell proliferation, lymph node status, vascular and lymphatic invasion, estrogen and progesterone receptor status, etc. (2). The increased incidence of breast cancer and its association with mortality highlight the need for a new therapeutic approach, especially targeted treatment (3). The human epidermal growth factor gene (HER2) located on the long arm of chromosome 17 is a very important oncogene in breast cancer (4). Many studies have shown the overexpression of this oncogene in 20% - 30% of breast cancer cases (5). The overexpression of the HER2 gene is associated with the rapid progression of the disease, increased metastatic potential, and tamoxifen resistance (6).

The discovery of appropriate targeted therapeutics against the HER2 gene (i.e., Herceptin) has led to great medical success in treating breast cancer (7). Herceptin

(trastuzumab) is a human anti-HER2 monoclonal antibody that targets the extracellular component of this oncogene. This drug (alone or in combination with chemotherapy) stops the progression of the disease and increases overall survival in HER2-positive breast cancers (8). Given the effectiveness of trastuzumab merely in HER2-positive patients, it is very important to diagnose the reliable status of HER2 positivity. In addition, trastuzumab is costly with serious side-effects on the heart. As a result, the HER2 status needs to be carefully determined (9).

The methods used to determine the HER2 status include gene assay (CISH, FISH, and PCR) and direct detection of the HER2 protein at the cell surface (Western blot and IHC). The most commonly used methods are IHC, CISH, and FISH (3). Since IHC is faster, easier, and more cost-effective than other methods, it is used as a routine test to check the HER2 status. The results of this test are reported as a four-propensity score (from 0 to 3+) based on the percentage of tumor-positive cells and color intensity. A score of 2+ in invasive breast cancer is considered to be intermediate. The intermediate results (accounting for approximately 15% of the cases) should be further examined by the FISH or CISH method, which is more accurate and more reliable than IHC.

2. Objectives

The aim of this study was to determine the equivocal HER2 gene expression in patients with invasive ductal carcinoma of the breast approved by the CISH or FISH method and its association with clinicopathologic variables.

3. Methods

This analytical, cross-sectional, census-based study was approved by the Local Ethics Committee and written informed consent was obtained from patients. Overall, out of 303 patients with invasive ductal cancer and HER2 2+ (based on IHC), 56 patients did not perform CISH or FISH for different reasons and thus, they were excluded from the study. Therefore, 247 patients were finally enrolled. The information was obtained by filling out questionnaires based on the patients' records or telephone contacts with them. By referring to the Pathology Lab Archive, all IHC results related to breast cancer patients were reviewed. All cases of invasive ductal cancer with HER2 2+ between 2011 and 2016 were identified for data extraction. Patients' information included age, tumor grade, the status of estrogen and progesterone receptors, and KI-67 status of the cases. The tumor grade (by evaluating tubule formation, degree of the nucleus, and mitotic rate) was recorded in three groups of patients including well differentiated (I), moderately differentiated (II), and poorly differentiated (III). Then, the results of the definitive tests, CISH or FISH, in patients with HER2 2+ were obtained from labs and oncologists' offices and through telephone calls to patients. The collected data were analyzed using SPSS (version 22) by the chi-square and Fisher's exact tests. The significance level was set at $P \leq 0.05$.

4. Results

In total, we found 303 cases of invasive ductal cancer with HER2 2+ (based on IHC). The results of the complementary CISH or FISH test were obtained from labs for 247 patients (56 cases did not perform CISH or FISH for different reasons). The mean age of 247 patients was 48.21 ± 11.33 years within the range of 23 to 77 years. There were 136 (55.1%) patients aged less than 50 years and 111 (44.9%) older than 50. Of 247 patients, 113 (45.7%) were estrogen receptor-negative and 134 (54.3%) were positive; 146 (59.1%) patients were progesterone receptor-negative and 101 (40.9%) were positive. Of the 247 patients, the Ki-67 status results were available for 199 patients of whom, 57 (28.6%) cases were negative and 142 (51.4%) were positive for this gene; the information of 48 patients (19.4%) was incomplete. Of the 247 patients, 193 patients had information related to the grade of the disease. Of the 193 patients, 3 (1.6%) were in grade I, 104 (53.9%) in grade II, and 86 (44.6%) in grade III; moreover, the information of 54 patients (21.9%) was incomplete.

The results of the complementary CISH test or FISH test were negative for 124 (50.2%) patients and positive for 123 (49.8%) patients. According to the chi-square test, there was a relationship between the age group (less than 50 and more than 50 years) and CISH results (P value < 0.05) (Table 1). Consequently, with an increase in age, the probability of CISH positivity decreased. According to Fisher's exact test, there was a significant relationship between the age groups and CISH (P value < 0.05). As a result, in the age group of fewer than 29 years, there was the most likelihood of CISH positivity and in the age group of 60, there was the most likelihood of CISH negativity (Table 2). According to the chi-square test, there was a significant relationship between the Ki-67 status and CISH results (P value < 0.05). As a result, with a positive Ki-67 result, the probability of positive CISH increased. According to the chi-square test, there was a significant relationship between ER (estrogen receptor) and CISH variables (P value < 0.05). As a result, the CISH result had an inverse relationship with the ER. In this way, with positive estrogen receptor, the probability of a positive result in CISH decreased. According to the chi-square test, there was no significant relationship between PR (progesterone receptor) and CISH variables (P value >

0.05). Based on Fisher's exact test, there was no significant relationship between the disease grade and CISH (P value > 0.05). According to the chi-square test, there was a significant relationship between ER and PR variables (P value < 0.05). As a result, patients who had estrogen receptor were more likely to have progesterone receptors and vice versa. According to the chi-square test, there was no significant relationship between age groups (less than 50 and more than 50) and Ki-67 status (P value > 0.05). According to the chi-square test, there was no significant relationship between ER and Ki-67 status (P value > 0.05). According to the chi-square test, there was no significant relationship between PR and Ki-67 status (P value > 0.05). Based on Fisher's exact test, there was no significant relationship between grade and Ki-67 status (P value > 0.05). Based on the *t* test, there was a significant difference between the mean age of patients in the group with positive and negative CISH. The mean age in the negative CISH group was higher than the mean age in the positive CISH group (50.2 years versus 46.2 years) (Table 3).

Table 1. Distribution of CISH Results in Patients with Invasive Ductal Carcinoma and Equivocal HER2 Gene Expression in the IHC Method by Age[a, b]

Age	CISH		Total
	Positive	Negative	
Less than 50 years	76 (55.9)	60 (44.1)	136 (100)
More than 50 years	47 (42.3)	64 (57.7)	111 (100)
Total	123 (49.8)	124 (50.2)	247 (100)

[a]Values are expressed as No. (%).
[b]P value = 0.034

Table 2. Distribution of CISH Results in Patients with Invasive Ductal Carcinoma and Equivocal HER2 Gene Expression in the IHC Method by Age Groups[a,b]

Age groups	CISH		Total
	Positive	Negative	
Less than 29 years	6 (75)	2 (25)	8 (100)
30 - 39 years	26 (51)	25 (49)	51 (100)
40 - 49 years	44 (57.1)	33 (42.9)	77 (100)
50 -59 years	35 (51.5)	33 (48.5)	68 (100)
60 - 69 years	9 (26.5)	25 (73.5)	34 (100)
More than 70 years	3 (33.3)	6 (66.7)	9 (100)
Total	123 (49.8)	124 (50.2)	247 (100)

[a]Values are expressed as No. (%).
[b]P value = 0.031

Table 3. Relationship Between Age in Patients with Positive and Negative CISH Results[a]

CISH	Number	Mean Age ± SD, y
Negative	124	50.2 ± 11.70
Positive	123	46.2 ± 10.57

[a]P value = 0.005

5. Discussion

It is important to evaluate the HER2/neu gene status in breast cancer. In this study, 49.8% of the patients who had equivocal HER2 expression in the IHC test were confirmed for the expression of this gene by the complementary CISH tests. This rate is similar to the finding of a study by Dowsett et al. (48%) (10). Musa et al. obtained a rate of 36.5% (11). The result of the present study is much higher than the rates obtained by Meijer et al. (26.4%) (12), Mehrazma et al. (20.8%) (13), Zhang et al. (29%) (14), Mostafa et al. (18%) (5), and Sinczak-Kuta et al. (10%) (2). This may be due to the type of the kit or antibody used, different boundaries used to interpret the coloring result, or the different interpretation of the IHC test results by pathologists. The way and the time of tissue preparation in both IHC and CISH/FISH tests can be quite effective, as well (13). Moreover, the heterogeneity of the tumor can also be the cause of different results (15). In this study, there was a correlation between the age group (less than 50 and more than 50) and the confirmation of HER2/neu overexpression by CISH. Thus, the chance of positive CISH decreased with increasing age. Most cases of positive CISH were found at the age of 29 years (75%). The age range of 60 to 69 years was associated with the highest probability of negative CISH (73.5%). In a study, the status of HER2/neu was associated with age and a lower age at the time of diagnosis was accompanied by the overexpression of the gene (16). However, in other studies, there was no significant relationship between age and HER2/neu amplification (5, 11). Moreover, the results of this study clarified an inverse relationship between estrogen receptor and overexpression of HER2/neu, which was similar to other studies (5, 7, 11). However, contrary to those studies, there was no significant relationship between progesterone receptor and overexpression of this gene in the current study. Our results indicated that with the positivity of the estrogen receptor, the probability of CISH positivity decreased. However, in the current study, the association between progesterone receptor positivity and FISH was not statistically significant. In this study, there was no significant relationship between tumor grade and CISH result, which is similar to other studies (5, 14, 17-19). Moreover, similar to other studies, there was a correlation between the tumor grade and the disease progno-

sis; the higher the score, the worse the prognosis (20). In the present study, we did not investigate the relationship between the stage of the disease and the amplification of HER2/neu gene expression, but in another study, these two variables had no meaningful relationship (5) and in Zhang et al. study, there was a significant correlation between the overexpression of HER2/neu gene in complementary tests and higher stages of the disease (14). In this study, there was a significant relationship between KI-67 and CISH results. By increasing the KI-67 score, the probability of a positive result in the CISH test increased. It is worthy to mention that this variable was not investigated in other studies while our study showed that this factor could also be considered a predictor of HER2/neu overexpression.

In conclusion, the significance of determining the status of the HER2/neu gene in breast cancer is well known. Given the numerous side-effects and high costs of Herceptin therapy, an equivocal IHC (2+) test requires a more accurate investigation by methods that are more costly and time-consuming than IHC. Our results showed that the frequency of false negative HER2 tests at our center is high. It is proposed to perform more precise complementary tests to determine the status of the HER2 gene by methods such as CISH in addition to performing IHC at the early stages. Moreover, laboratories should follow the ASCO/CAP guideline for HER2 evaluation in breast carcinoma.

5.1. Conclusions

Our results showed that false negative HER2 test was high in our sample. The cause may be due to the type of the kit or antibody used or different guidelines used for the interpretation of the test results. It is also proposed to perform more precise complementary tests to determine the status of this gene. Moreover, laboratories should follow the ASCO/CAP guideline for HER2 evaluation in breast carcinoma.

Footnotes

Authors' Contribution: Fariba Binesh designed study. Amirmasoud Homayon and Hassanali Vahedian Ardekani collected data. Sedighe Vaziribozorg performed data analysis. Seyed Hossein Shahcheraghi wrote article.

Conflict of Interests: The authors declare that they have no conflicts of interest.

Ethical Approval: The study protocol was in accordance with the latest Declaration of Helsinki for medical research involving human subjects and was approved by the Local Ethics Committee.

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Patient Consent: Informed consent was obtained from all participants of the study.

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