

Comparison of two different methods used in the assay of Her 2/Neu (C-erb B2) in breast cancer

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Abstract

Aim: To investigate the comparison of two different methods used in the assay of Her 2/Neu (C-erb B2) in patients with breast cancer.

Methods: Hospital records of 150 cases diagnosed with breast cancer between 2010-2012 at the Oncology Clinic of Sakarya Hospital, Turkey, were retrospectively evaluated. The paraffin blocks obtained from the pathology laboratory were examined. In these patients, Fluorescence in situ hybridization (IHC) and Fluorescence in situ hybridization (FISH) methods were compared in the assay of Her 2/Neu. The number of the patients in whom IHC and FISH were negative and IHC and FISH were positive were identified.

Results: FISH was determined to be negative in all of the 112 patients in whom Her 2/Neu was determined to be negative through the IHC method. FISH was determined to be positive in 19 (95%) out of 20 patients in whom Her 2/Neu 2+ and 3+ were detected through the IHC method. FISH was determined to be positive in 11 (61.1%) out of 18 patients in whom Her 2/Neu 1+ was detected through the IHC method.

Conclusion: The results of this present study suggest that FISH method seems to be superior to IHC in determining the overexpression of Her 2/Neu. Besides, determining the overexpression of Her 2/Neu is of great importance in terms of the trastuzumab treatment in the patients with advanced-stage breast cancer who are not recommended to have a surgical resection.

Keywords: breast cancer, Her 2/Neu, fluorescence in situ hybridization, immunohistochemistry, trastuzumab.

Introduction

Breast cancer, which is the most prevalent malignancy throughout the world, is the second leading cause of death in women. HER-2/neu oncogene located in the chromosome 17q is the proto-oncogene which encodes the receptor of epidermal growth factor or the transmembrane tyrosine kinase growth factor receptor belonging to HER family (1,2). Due to the gene amplification 95% of the time (3,4), HER2/neu overexpression is observed in 20-30% of the cases suffering from breast cancer. Slamon DJ et al. were the first authors to discover a statistically strong and significant correlation between gene amplification and the periods of disease relapse and survival (5). Transtuzumab, as a monoclonal antibody, was powerfully positive in HER-2/neu and is effective in the patients with metastatic disease who don't respond to the chemotherapy treatment (1,6). There are a number of methods in determining HER-2/neu gene status in the tumour tissues. Immunohistochemistry (IHC) is the method used most frequently in the evaluation of gene overexpression. Fluorescence in situ hybridization (FISH), on the other hand, is a more recent technique which detects gene amplification in tumour tissues. Both techniques can be applied to formalin-fixed and paraffin-embedded tissues. The evaluation of HER-2/neu status through IHC is a more simple and practical method easily performed with lower costs in all the pathology laboratories. Yet, IHC testing may lead to several problems resulting from diverse antibodies, tissue processing and the interpretation of the results in various ways. FISH is quite an accurate method with a perfect sensitivity and specificity in determining HER-2/neu gene amplification. It has also a low interlaboratory variability thanks to a standardized threshold used for determining HER-2/neu gene amplification (7-9). Each detection performed on HER-2/neu gene amplification by using the FISH method is considered to be the best indicator to begin the transtuzumab treatment in the patients with invasive breast carcinoma (10). The

FISH method is not preferred as a technique in many pathology laboratories due to its more technical and costly qualities (1). Our primary objective in this study was to compare the expression and amplification values of HER-2/neu gene between IHC and FISH methods.

Methods

In this present study, the hospital records of 150 cases diagnosed with breast cancer between 2010-2012 at the Oncology Clinic of Sakarya Hospital, Turkey, were retrospectively scanned. The paraffin blocks obtained from the pathology laboratory were examined.

In these patients, IHC and FISH methods were compared in the assay of Her 2/Neu. The number of the patients in whom IHC and FISH were negative and IHC and FISH were positive were identified.

All the analyses were performed using the SPSS for Windows (version 21,0; SPSS/IBM).

Results

The hospital records of 150 patients with breast cancer were documented retrospectively. 68 (45.3%) of the patients were premenopausal and 82 (54.7) of them were postmenopausal. The complete instruments for operation consisted of the Modified Radical Mastectomy (MRM) materials. 19 of the patients (12.7%) used OCPs (oral contraceptives), 6 (4%) of them had HRT (hormon replacement therapy), 13 of them (8.7%) had smoking habits and 1 of them (0.7%) consumed alcohol. The number of patients with medical record of breast cancer in their families was 13 (8.7%). The number of patients with the estrogen receptor positivity was determined to be 100 (66.7), whereas the number of those with the progesterone receptor positivity was 87 (58%), the number of patients with HER 2/Neu positivity was 30 (20%) (Table 1). A significant difference was found when the results of FISH and immunohistochemical Her 2/Neu were compared in the cases within our study group. FISH was found to

be negative in 112 cases in whom the immunohistochemical Her 2/Neu was evaluated as negative. FISH was positive in 11 out of 18 IHC positive patients, whereas FISH was positive in 3 out of 4

IHC positive patients, and FISH was evaluated as positive in all of the 16 patients in IHC positive patients (Table 2).

Table 1. Demographic, clinical and laboratory findings

Parameters	%
Premenopausal Patient	45.3
Postmenopausal Patient	54.7
Use of OCP	12.7
Use of HRT	4
Smoking	8.7
Alcohol Consumption	0.7
Breast Cancer within the family	8.7
Estrogen Receptor Positivity	66.7
Progesterone Receptor Positivity	58
Her 2 /Neu positivity	20

Table 2. Comparison of IHC and FISH Methods

IHC method	FISH method	Similarity %
Her 2/Neu negative patients number: 112	112 FISH -	100%
Her 2/Neu 1 + patients number: 18	11 FISH +	61%
Her 2/Neu 2 + patients number: 4	3 FISH +	75%
Her 2/Neu 3 + patients number: 16	16 FISH +	100%

Discussion

The results of this present study suggest that FISH method seems to be superior to IHC in determining the overexpression of Her 2/Neu. Besides, determining the overexpression of Her 2/Neu is of great importance in terms of the trastuzumab treatment in the patients with advanced-stage breast cancer who are not recommended to have a surgical resection. Trastuzumab (monoklonal anti-HER2 antibody) has been used in the treatment of breast cancers where HER2 receptor is over-expressed (11). There were numerous patients with the overexpression of HER2 protein and/or HER/2neu

gene amplification who had benefited from the Trastuzumab treatment (12). In our study, FISH was determined as negative in all of the 112 patients in whom Her 2/Neu was negative by means of the IHC method. FISH was determined to be positive in 19 (95%) out of 20 patients in whom Her 2/Neu 2+ and 3+ were detected by means of the IHC method. FISH was determined to be positive in 11 out of 18 patients (61.1%) in whom Her 2/Neu 1+ was detected through the IHC method. In a number of studies in the literature, it is stated that with IHC there is no need

for 0 and +1 cases to be verified by means of the FISH method. However in this present study, it was concluded that +1 and +2 cases in particular required to be verified through the FISH method. In a study conducted in this matter, the FISH positivity rates in the cases immunohistochemically scored as 0,+1,+2 and +3 were found as 3.5%, 6.4%, 25.7%, and 81.5%, respectively. According to these results, considering the trastuzumab treatment, it was concluded that the FISH method could be applied to 0 and +1 cases. The fact that the amplification rate determined in +3 cases was 81.5% suggested that the FISH method in this group of cases does not provide further information. It could be inferred from the literature that the FISH was a much more reliable method than the IHC method in determining the HER2/neu status and that more sophisticated measurements for quality control were required for more accurate immunohistochemical results (13,14). In the literature, the

major reason for the discordance between FISH and IHC is considered in those +2 cases. In our study, an amplification by FISH was determined in 75% of the cases in whom +2 was found through the IHC. The concordance between FISH and IHC in +2 cases have been reported as 20-30% in the literature. Our findings in +2 cases were the same to the literature, in addition we could say that the cases in this group required to be confirmed through the FISH method. The amplification by FISH is positive in 100% of +3 cases by IHC. In the literature, the amplification by means of FISH is reported to be at a rate of 79-100% in +3 cases. According to the literature there is no need for +3 cases to be confirmed through the FISH method. In conclusion, it was inferred from this present study that FISH method should be applied in +1 and +2 cases detected by the IHC method. However, FISH method isn't necessary to be applied in +3 cases detected by the IHC method.

Conflicts of interest: None declared.

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