Clinical Research

Are Fibrocystic Changes Innocent? Importance of HER-2 in Fibrocystic Changes of Breast and Correlation Breast Cancer Risk

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ABSTRACT

Objective: Fibrocystic change refers to nonproliferative lesion. Determining whether this lesion is a risk factor for developing breast cancer has been one of the most important issues. In order to determine this risk, we used HER-2 by immunohistochemistry in this study.

Material and Method: 163 mastectomy materials which had tumor diagnosis between 1995 and 2016 in our clinic and had findings of fibrocystic changes in non-tumor areas, and 151 excisional breast biopsy materials which had diagnosis of fibrocystic change but had not malignant mass were included into the study. The tissues containing fibrocystic changes in both groups were stained with HER-2 by immunohistochemical methods.

Results: It was found that HER-2 staining was more frequent in FCC areas with in mastectomy cases due to tumor ($\chi 2 = 37{,}079$; p =,000).

Conclusions: We found out that HER 2 staining was significantly more positive in fibrocystic change cases with in mastectomy cases due to tumor than non-malignant cases and that the risk of malignancy was higher in HER-2 positive cases. We believe that HER-2 staining for breast biopsies will be helpful for determining malignancy potential in benign lesions of breast.

***Keywords**: HER-2, Breast, Fibrocystic Change.*

ÖZET

Fibrokistik Değişiklikler Masum mudur? Memenin Fibrokistik Değişikliklerinde HER-2'nin Önemi ve Meme Kanseri ile İlişkisi

Amaç: Fibrokistik değişiklik nonproliferatif bir lezyondur. Bu lezyonun meme kanseri gelişiminde bir risk faktörü olup olmadığını belirlemek en önemli konularından biri olmuştur. Bu riski belirlemek için, bu çalışmada immünohistokimyasal olarak HER-2 kullanılmıştır.

Gereç ve Yöntem: Kliniğimizde 1995-2016 yılları arasında tümör tanısı alan ve tümör olmayan bölgelerde fibrokistik değişiklikleri olan 163 mastektomi materyali ile fibrokistik değişiklik tanısı konan ancak malign kitlesi olmayan 151 eksizyonel meme biyopsisi materyali çalışmaya alındı. Her iki gruptaki fibrokistik değişiklikleri içeren dokular HER-2 ile immünohistokimyasal yöntemlerle boyandı.

Bulgular: Tümör nedeniyle mastektomi yapılan olguların tümör dışı fibrokistik alanlarında HER-2 boyanmasının anlamlı derecede yüksek olduğu saptandı ($\chi 2 = 37{,}079$; p=,000).

Sonuç: HER -2 boyamasının tümör nedeniyle mastektomi yapılan olgularda tümör dışı alanlarda fibrokistik değişim gösteren dokularında malign olmayan olgulara kıyasla anlamlı olarak daha pozitif olduğunu ve HER-2 pozitif olgularda malignite riskinin daha yüksek olduğunu tespit ettik. Meme biyopsileri için HER-2 boyanmasının meme benign lezyonlarında malignite potansiyelinin belirlenmesine yardımcı olacağına inanıyoruz.

***Anahtar Sözcükler:** HER-2, Meme, Fibrokistik Değişiklik*

Fibrocystic change (FCC) refers to nonproliferative lesion. FCC occurs with great frequency in the general population. It affects women between the ages of 25 and 50 years and it is rare below the age of 20. When symptomatic, patients suffer from breast tenderness, swelling, pain and may also complain of menstrual abnormalities. Hormonal abnormalities that have been caused in the pathogenesis of these changes include a relative excess of estrogen over progesterone, hyperprolactinemia, and increased thyroid hormone activity (1- 6).

The HER-2/neu protooncogene known as C-erbB-2 is localized in the short arm of chromosome 17 (17q21). This gene encodes transmembrane HER-2 glycoprotein with a molecular weight of one hundred and eighty five kilodaltons (kDa) and comprising of 1255 amino acids. Normal breast epithelial cells contain 2 copies of the HER-2 gene and express low levels of HER-2 receptor on the cell surface. However, in some individuals, the number of gene copies in the cell is increased due to oncogenic transformation and gene amplification results in increased membrane expression of HER-2 protein. For this reason, the basic mechanism for HER-2 protein overexpression is assumed to be gene amplification. Increased membrane expression of HER-2 protein activates HER-2 tyrosine kinase by increasing dimerization. The transmission of this activation signal to the cell nucleus causes cell replication and mitosis in the cell undergoing oncogenic transformation. Thus, HER-2 gene amplification is considered to contribute to the developmental process of breast carcinoma, i.e. carcinogenesis (7- 9).

Over the years, it has been one of the major issues to determine whether these lesions are a risk factor for the subsequent development of breast cancer (10). In this study, we aimed to answer the question "Can we predict the malignancy potential of patients with fibrocystic changes using the HER-2 gene?"

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MATERIAL AND METHOD

This study is a retrospective research study. After approval of regional ethics committee, 163 mastectomy materials (group 1), which had tumor diagnosis between 1995 and 2016 in our clinic and had findings of fibrocystic changes in non-tumor areas, and 151 excisional breast biopsy materials (group 2) which had diagnosis of fibrocystic change and accessible in pathology archives were included into the study. Since none of the Group 2 cases should have a later developing tumor, those who later received a tumor diagnosis, atypia and proliferative changes were excluded from the study. Hematoxylin eosin stained sections from both group 1 and group 2 were re-evaluated under light microscopy. The tissues containing fibrocystic changes in both groups were stained with HER-2 by immunohistochemical methods.

For immunohistochemical staining, sections with a thickness of 4µm were taken from parafin blocks with the help of Leica RM 2255 microtome to be mounted onto poly-L-lysin coated slides. After deparaffinization and rehydration, the sections were soaked in 3% H2O2 for 15 minutes to block endogenous peroxidase activity and then protein blockage was performed. HER-2 was incubated for 1 hour with its primary antibody, and then it was incubated with secondary antibody binding to the primary antibody, and streptavidin- biotin- peroxidase enzyme complex for 15 minutes. It was washed with a buffer solution at each stage. The evaluation was made by a single pathologist under light microscopy. According to the recommendations of the American Society of Clinical Oncology / College of American Pathologists (ASCO / CAP), it was scored as follows:

Score 0: No membrane staining

Score 1: Whatever the rate in invasive carcinoma cells is, there is poor staining in non-membranous staining which is not surrounding the entire membrane and are difficult to identify, or poor staining which surrounds the entire membrane in less than 10% of cells. Immunohistochemistry refers to negative, in situ hybridization is not recommended.

Score 2: The membranous staining which completely surrounds the cytoplasmic membrane in at least 10% of invasive carcinoma cells but has moderate intensity, or the membranous staining which is less than 30% but has strong intensity is observed. It indicates a suspicious positive immunohistochemically, it should be confirmed by in situ hybridization.

Score 3: Strong uniform immunostaining which surrounds the entire cytoplasmic membrane in at least 30% of invasive carcinoma cells is seen. It is strong positive immunohistochemically, in situ hybridization is not recommended (11).

Statistical Analysis

All analyses were performed with the Statistical Package for Social Sciences (SPSS) for Windows 17.0 program. For relationship between FCC and risk of malignancy in this study we were performed with Pearson's $\chi 2$ test.

RESULTS

As a result of HER-2 staining applied to the specimens of group 1 and group 2 by immunohistochemical methods; in group 1 cases, score 3 was detected in 7 cases. Others were considered negative. In the samples belonging to Group 2, only one case had a score 1 staining and the other 150 cases were evaluated as score 0. According to these values, there was a significant difference between group 1 and group 2 in terms of staining scores and it was found that HER-2 staining was more frequent in FCC areas with tumors, ($\chi 2$ = 37,079; p =,000) (Table 1).

Table 1. *Results of Chi-Square Test for Differences in staining between group 1 and group 2.*

Variables	n	Degrees of Staining		$\chi 2$	*p*
		Negative	Positive		
Group 1	163	156	7	37,079	,000
Group 2	151	151	0		

Of the 163 tumor tissues of Group 1, 93 were diagnosed with invasive carcinoma NST, 57 were diagnosed with invasive lobular carcinoma, 4 were diagnosed with intraductal papillary carcinoma, 4 were diagnosed with medullary carcinoma, 3 were diagnosed with mucinous carcinoma and 2 were diagnosed with metastatic carcinoma. Bi-directional chi-square test was used to reveal the significant difference between tumor types with FCC and staining scores. It was found that there was no significant difference between tumor types with FCC and staining scores ($\chi 2$ = 11,612; p =0.708). Results related to the analyses are given in Table 2.

In areas of fibrocystic changes positive staining was detected in the cyst epithelium of 4 cases and in ductal adenosis areas in 3 cases.

Table 2. *Chi-Square Test Results for the Difference between Tumor Types and Staining Degrees IC-NST: Invasine Carcinoma NST, ILC: Invasive Lobuler Carcinoma, IDPC: Intraductal Papillary Carcinoma, MUC: Mucinous Carcinoma, MEC: Medullary Carcinoma, MTC: Metaplastic Carcinoma.*

		Degrees of Staining					
FC with Tumor types	**n**	**Score 0**	**Score 1**	**Score 2**	**Score 3**	$\chi 2$	*p*
FC with IC-NST	93	68	14	7	4	11,612	0,708
FC with ILC	57	46	4	5	2		
FC with IDPC	4	2	0	1	1		
FC with MUC	3	3	0	0	0		
FC with MEC	4	4	0	0	0		
FC with MTC	2	2	0	0	0		

DISCUSSION

The term benign breast disease (BBD) is used to describe a combination of several clinical diagnoses noted at breast biopsy. In 1985, the Cancer Committee of the College of American Pathologists reached a consensus on the type of pathologic findings included in BBD and

on the grouping of the pathologic diagnoses into categories relative to the degree of invasive breast cancer risk likely to be associated with each category (12). In 1998, Fitzgibbons et al. (13) reported an updated version of the consensus definitions. Several authors have studied the risk of breast cancer associated with BBD, however, none of these authors has fully explored the independence of BBD in breast cancer risk from the known breast cancer risk factors (14- 21).

There are different opinions on whether fibrocystic changes are related to malignancy (22). Fibrocystic changes are found in the lower-category benign breast disease (LC-BBD) group according to the classification of the Cancer Committee of the College of American Pathologists, and in this group of diseases (Adenosis, ductal ectasia, fibroadenoma without complex features, fibrosis, mastitis, mild hyperplasia without atypia, ordinary cysts, simple apocrine metaplasia, squamous metaplasia) increased risk of malignancy has not been reported (12, 13). Likewise, Moinfar (23) reported in his book Essentials of Diagnostic Breast Pathology that fibrocystic disease is not associated with increased risk of cancer. On the other hand, in a study conducted on 11307 female patients by Wang et al. (16), LC_BBD was diagnosed in 1376 cases and cyst was detected in 674 of them. Invasive breast carcinoma developed in 264 of 674 patients with cyst diagnosis and risk of developing malignancy for cysts was determined as 1.79, while this risk was reported to be 1.42 for other LC-BBD. An elevated risk of breast cancer associated with a diagnosis of a cyst has been indicated by studies of case series (24- 26) In a cytogenetic analysis of 69 FCC cases and 10 normal breast tissues by Lundin et al. (27), clonal chromosome aberration was detected in 6 FCC cases, but all normal tissues were found to have normal karyotypes. The frequency of chromosomal abnormalities found in BBD, although lower than in breast carcinoma, correlates with the corresponding risk of developing invasive carcinoma.

The molecular identification of high risk breast lesions could improve the effect of current preventive strategies and the application of selected therapeutic interventions (28, 29). According to the field cancerization hypothesis, as widely reported in head and neck cancer (28, 30, 31), it is likely that benign breast tumors, precursors for invasive cancer and the normal appearing peritumoral tissue, may harbor molecular changes heralding early stages of cancer development (27, 32). Moreover, the benign parenchyma of cancer-containing breasts and the contralateral normal epithelium in patients who experienced cancer in one breast, can share the same pattern of chromosomal abnormalities with invasive carcinoma (33).

HER-2 gene amplification is considered to contribute to the development of breast carcinoma, i.e. carcinogenesis (13- 15). In a HER-2 study performed by Rohan (34) et al to determine the risk of cancer, HER-2 staining was performed by immunohistochemical methods on 74 female patients who were diagnosed with BBD and who subsequently developed malignancy and 309 female patients who did not develop malignancy, and HER-2 staining was not associated with malignancy development. In a study by Gusterson et al. (35) on 150 BBDs, none of the 22 FCC cases was reported for HER2 staining.

In this study that we aim to determine the risk of malignancy development in patients with fibrocystic changes using the HER-2 gene, it was found that there was a significant difference in the degree of staining in areas of FCC with breast tumor cases. HER-2 was positive in 7 of the FCC cases with malignancy, but no HER-2 positivity was found in any of the FCC without breast carcinoma. There was a significant difference between FCC with breast tumor cases and without tumor in terms of staining scores and it was found that HER-2 staining was more frequent in FCC areas with tumors (χ^2= 37,079; p <0,05).

Ordinary apocrine cells are an important constituent feature of FCC. Several studies have analyzed the possible relationship between apocrine change in FCC and breast carcinoma. Thus, Foote and Stewart in a study of 300 breast cancer specimens and 200 noncancerous specimens, analyzed the frequency of apocrine epithelium (36). They found no significant difference in the frequency of apocrine change. Their conclusion was that apocrine metaplasia is unlikely a precursor of breast cancer, given the high prevalence of this feature. Furthermore, Wellings and Alpers (37) in their subgross analysis of 186 autopsy breast tissue specimen and 107 breast cancer specimens, failed to demonstrate a continuous spectrum of apocrine metaplasia to overt carcinoma. However, in this study, no staining was observed in apocrine metaplasic areas. In areas of fibrocystic, positive staining was detected in the cyst epithelium in 4 of the samples, in ductal adenosine areas in 3 of the samples. We were convinced that apocrine metaplasia has a lower risk of malignancy than cyst formation and ductal adenosis.

In this study that we conducted on FCC with tumor cases and FCC without malignancy. The number of cases was relatively low. Performing these studies in larger groups will increase their significance. In this study, we found out that HER 2 staining was significantly more positive in FCC with malignancy than without malignancy and that the risk of malignancy was higher in HER-2 positive cases by immunohistochemistry. Early diagnosis is very important in breast carcinoma. Screening programs in our country are quite common. However, these programs pose a financial burden. We therefore believe that HER-2 staining for breast biopsies will be helpful for determining malignancy potential in FCC of the breast, and that positive staining will be critical about adjustment of patient follow-up frequency according to this potential.

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