JBUON 2015; 20(1): 45-49
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Neoadjuvant chemotherapy-induced changes in immunohistochemical expression of estrogen receptor, progesterone receptor, HER2, and Ki-67 in patients with breast cancer

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Summary

Purpose: *The impact of neoadjuvant chemotherapy (NACT) on immunohistochemical markers in breast cancer specimens remains controversial. We designed the current study to investigate the potential changes in estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 expression before and after NACT in a cohort of Turkish patients with breast cancer.*

Methods: *This research was designed as a prospective, observational study of 100 consecutive patients with breast cancer (mean age 47.8±11.4 years) who were scheduled to undergo anthracycline- and/or taxane-containing NACT before attempting cytoreductive surgery at the Department of Oncology of the Uludag University Medical Center, Bursa, Turkey. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded specimens.*

Results: *Changes in immunohistochemical markers before and after NACT were only significant for HER-2 and Ki-67. More specifically, the number of HER-2-positive specimens decreased from 21 before NACT to 8 after NACT ($p<0.001$). Similarly, the number of tumor samples positive for Ki-67 decreased significantly from 65 to 24 after NACT ($p<0.001$). Mean pre- and post-treatment tumor grades of differentiation before and after NACT were 2.56 ± 0.67 and 2.37±1.07, respectively ($p<0.05$). We did not find any significant associations between baseline ER, PR, HER2, and Ki-67 expression with both overall survival (OS) and disease-free survival (DFS).*

Conclusion: *Our study suggests that NACT reduces the expression of HER2 and Ki-67 in breast cancer specimens. The significance of NACT-induced changes in the immunohistochemical expression of HER2 and Ki-67 in patients with breast cancer should be further studied in future translational and clinical research.*

Key words: *breast cancer, estrogen/progesterone receptor, HER2, immunohistochemistry, Ki-67, neoadjuvant chemotherapy*

Introduction

Breast cancer is the most common cancer in women worldwide, comprising 23% of all malignancies in females [1]. According to the American Cancer Society, about 1.3 million women are diagnosed with breast cancer annually around the world and approximately 465,000 cases will die from this disease [2]. NACT is defined by the administration of chemotherapy before locoregional treatment (with surgery and/or irradiation) [3]. NACT followed by cytoreduction has currently become a part of standard care for patients with locally advanced breast cancer [4]. From a clinical standpoint, NACT can offer several advantages, including downstaging of large tumors, providing information on tumor response to a specific chemotherapeutic agent or agents, and improving clinical outcomes (presumably through early clearance of systemic micrometastates) [5-7].

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Received: 09/08/2014; Accepted: 01/09/2014

ER and PR are hormone activated nuclear transcription factors that influence directly the mammary epithelial growth, differentiation, and survival [8,9]. Hormone receptor status assessed by means of immunohistochemistry has been identified as an independent predictor of therapeutic response [10,11]. In particular, evidence suggests that ER-negative tumors tend to respond better to chemotherapy than ER-positive tumors [12]. Similarly, immunohistochemical overexpression of HER2 – a member of the epidermal growth factor receptor family of tyrosine kinase receptors [13] – has been related to poor outcomes, although its potential association to the response to NACT remains unclear [14]. Ki-67, a cell cycle and a mitosis-related marker, is a nonhistone nuclear protein that is closely linked with cell proliferative activity [15]. In a sample of 148 patients with breast cancer, Nishimura et al. [16] have previously shown that reduced immunohistochemical expression of Ki-67 before NACT is significantly associated with its clinical effectiveness. Moreover, Ki-67 expression was found to be significantly reduced after NACT and associated with both clinical response and DFS [16]. However, the effect of NACT on breast cancer immunohistochemical markers remains controversial. In this regard, Lee et al. [17] have reported changes in ER and PR expression in 61% of patients undergoing NAC. However, Arens et al. [18] did not find any significant impact of NACT on ER, PR, and HER2 expression. Moreover, Kasami et al. [19] found significant modifications in PR expression, whereas no effect on ER and HER2 was identified. Similarly, Pedrini et al. [20] reported no significant differences in ER and PR expression before and after NACT.

Given the controversial findings on the impact of NACT on immunohistochemical markers in breast cancer specimens, we designed the current study to investigate the potential changes in ER, PR, HER2, and Ki67 expression before and after NACT in a cohort of Turkish patients with breast cancer.

Methods

Study participants

This research was designed as a prospective, observational study of 100 consecutive patients with breast cancer (mean age: 47.8±11.4 years) who were scheduled to undergo NACT before attempting cytoreductive surgery at the Department of Oncology of the Uludag University Medical Center, Bursa, Turkey. Enrollment was performed between September 2006 and August 2011. All patients were of Turkish descent. The clinicopathological characteristics of the study participants were collected from pathological reports and medical charts. Lesion staging was performed according to the sixth edition of the American Joint Committee on Cancer (AJCC) staging manual for breast cancer. All participants received anthracycline- and/or taxane-containing NACT before surgery.

The primary endpoint of the study was the change in the immunohistochemical expression of ER, PR, HER2, and Ki67 before and after NACT. The study protocol conformed to the principles of the Declaration of Helsinki and was approved by the local ethics committee. Before the study, each participant was informed about the purpose of the study and signed the informed consent form.

Immunohistochemical markers

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded breast cancer specimens as described previously [21]. ER and PR status were considered as positive if more than 10% of tumor cells showed staining. Immunohistochemical score of 3+ for HER2 was accepted as HER2 positivity. The immunohistochemical detection of Ki-67 (clone MIB-1, DAKO M7240, Dako Corporation, Carpinteria, CA, USA; dilution 1:70) was carried out as previously reported [21]. Ki-67 positivity was defined in presence of more than 15% positively stained cells.

Statistics

The data were checked for normality using the Kolmogorov-Smirnov test for continuous variables. Normally distributed variables were expressed as means and standard deviations, whereas skewed variables were given as medians and ranges. Categorical variables were assessed as counts and percent frequency and compared using the chi-squared test. All calculations were performed using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA). P values < 0.05 (two-tailed) were considered statistically significant.

Results

The general characteristics of the study patients are shown in Table 1. Of the 100 study participants, 90 (90%) had invasive ductal carcinoma, 6 (6%) invasive lobular carcinoma, and the remaining 4 (4%) other forms of carcinoma. The primary tumor status was T1 in 16 (16%) patients, T2 in 64 (64%), T3 in 12 (12%), and T4 in 8 (8%) patients. The lymph node status was as follows: N0 in 63 (63%), N1 in 35 (35%), N2 in 1 (1%) and N3 in 1 (1%) patient. The mean number of NACT cycles was 5.7±1.0. The patients were followed up for a mean of 38.2±11.4 months. In the entire study co-

Table 1. Clinicopathological characteristics of the 100 study participants

Characteristics	N (%)
Age (years)	47.8 ± 11.4*
Postmenopausal status (yes/no)	55/45
Preoperative primary tumor stage	
T1	16 (16)
T2	64 (64)
T3	12 (12)
T4	8 (8)
Preoperative axillary lymph node stage	
N0	63 (63)
N1	35 (35)
N2	1 (1)
N3	1 (1)
Number of nodal metastases	
No metastases	77 (77)
1-3	18 (18)
4-9	1 (1)
≥ 10	(1)
Unknown	(3)
Clinical TNM stage	
1A	13 (13)
1B	0 (0)
2A	48 (48)
2B	29 (29)
3A	5 (5)
3B	4 (4)
3C	1 (1)
Histology	
Invasive ductal	90 (90)
Invasive lobular	6 (6)
Mucinous	2 (2)
Tubulolobular	1 (1)
Unknown	1 (1)
Neoadjuvant chemotherapy	
Anthracycline combinations	40 (40)
Taxane/anthracycline combinations	48 (48)
Unknown	12 (12)
Number of chemotherapy cycles*	5.7 ± 1.0
Postoperative tumor size (mm)	18 (5-30)§
Postoperative primary tumor stage	
T1	62 (62)
T2	28 (28)
T3	10 (10)
T4	0 (0)
Pathological TNM stage	
0	13 (13)
1A	33 (33)
1B	1 (1)
2A	33 (33)
2B	17 (17)
3A	2 (2)
3B	0 (0)
3C	1 (1)

*mean±SD, §range

hort, the mean PFS was 36.5±12.1 months, whereas the mean OS was 37.1±11.3 months. The changes in ER, PR, HER2, and Ki-67 expression before and after NACT are displayed in Table 2. Changes in immunohistochemical markers before and after NACT were significant only for HER2 and Ki-67. More specifically, the number of HER2-positive specimens decreased from 21 before NACT to 8 after NACT ($p<0.001$). Similarly, the number of tumor samples positive for Ki-67 decreased significantly from 65 to 24 after NACT ($p<0.001$). Mean pre- and post-treatment tumor grades of differentiation before and after NACT were 2.56 ± 0.67 and 2.37 ± 1.07, respectively ($p<0.05$). We did not find any significant associations between baseline ER, PR, HER2, and Ki-67 expression with both OS and DFS (data not shown). Furthermore, ER, PR, HER2, and Ki-67 expression at baseline did not predict response to NACT.

Discussion

In general, NACT shows a recognizable impact on the immunohistochemical expression of different tumor biomarkers in breast cancer specimens [16-20]. However, the extent of such changes and the question as to whether such changes have an impact on tumor behavior and prognosis remain open. The results of this study conducted in Turkish patients with breast cancer provide evidence that NACT significantly reduces the immunohistochemical expression of HER2 and Ki-67 but not of ER and PR. The lack of impact of NACT on ER and PR expression observed in our report is in line with the results of two previous independent investigations [18,20]. However, it should be noted that Taucher et al. [22] reported significant differences in ER and PR expression before and after NACT, whereas a study by Kasami et al. [19] observed a significant change only for PR but not for ER. Such apparent discrepancies may be attributed, at least in part, to the different types of drugs used for NACT. As in the study by Pedrini et al. [20], in this report we used anthracycline- and taxane-based combinations. Taken together, these

Table 2. Changes in ER, PR, HER2, and Ki67 expression before and after neoadjuvant chemotherapy (N=100)

	Positive ER		*Positive PR*		*Positive HER2*		*Positive Ki-67*	
	Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Number of positive subjects	75	67	58	42	21	4*	65	24*

*p < 0.001

results suggest that such regimens do not seem to be able to induce significant differences in ER and PR expression between the initial biopsy and the tumor remaining after NACT. Consequently, anthracycline- and taxane-based combinations cannot select specific cell lines that express ER or PR. In any case, another potential reason for the discrepancies among studies may be related to methodological differences for assessing hormonal status (including immunohistochemistry vs biochemical assays), different sampling methods, and the absence of well-defined standard laboratory procedures [10,11].

In our study, we demonstrated that anthracycline- and taxane-based combinations used for NACT significantly reduced both HER2 and Ki-67 expression. Overexpression of HER2 – a 185-kDa protein receptor with tyrosine kinase activity and extensive homology to the epidermal growth factor receptor – may play a role in determining the aggressiveness of breast cancer and has been associated with poor outcomes [13,14]. In line with the results of previous studies [20,23], we found significant changes in HER2 expression after NACT. However, our findings are different from those reported by Arens et al. [18], who did not find any differences in HER2 receptor expression after NAC. In any case, the NSABP (National Surgical Adjuvant Breast and Bowel Project) B11 trial demonstrated that anthracyclines can allow HER2-overexpressing patients achieving similar survival rates with HER2-negative subjects [24]. Taken together, these data suggest that HER2 overexpression may be a marker of response to anthracycline-based chemotherapy. Moreover, our observation that the positive Ki-67 tumors dropped from 65 to 24% after NACT is in line with previous studies [16,18] and suggests that chemotherapy can exert a significant anti-proliferative effect on breast cancer cells. In particular, anthracycline-induced DNA damage may decrease the viability of newly formed cancer cells and reduce highly proliferating subclones of tumor cells.

There are several limitations in this study which need to be mentioned. First, this study was conducted in Turkish individuals, so results cannot be simply extrapolated to populations with different racial backgrounds. Second, our study should be considered as an exploratory analysis and independent replication is needed to extend and confirm our results. Moreover, breast cancer patients were treated on an individual basis according to each patient's disease characteristics based on clinical trial data and influenced by the personal experience of the medical oncologist.

These caveats notwithstanding, the results of the present study suggest that NACT reduces the expression of HER2 and Ki-67 in breast cancer specimens. The significance of NACT-induced changes in immunohistochemical expression of HER2 and Ki-67 in patients with breast cancer should be further studied in future translational and clinical research.

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