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ORIGINAL ARTICLE

Evaluation of changes in biologic markers ER, PR, HER 2 and Ki-67 index in breast cancer with administration of neoadjuvant dose dense doxorubicin, cyclophosphamide followed by paclitaxel chemotherary

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Summary

Purpose: *The aim of this study was to assess the changes in biologic markers of breast cancer ER, PR, HER 2 and Ki-67 in locally advanced breast cancer patients after neoadjuvant chemotherapy.*

Methods: *: Data from 63 locally advanced breast cancer patients (stage II or III), whose histological diagnosis was made by core biopsies were retrospectively evaluated. The patients were given 4 cyles of 600 mg/m^2 cyclophosphamide, 60 mg/m^2 doxorubicin every 15 days followed by 4 cycles of paclitaxel 175 mg/m^2 every 15 days, and they underwent surgery within two weeks after the last chemotherapy cycle. Expressions in the preoperative and postoperative status of ER, PR, HER 2 and Ki-67 were compared.*

Results: *The patient mean age was 49.2 ±10.7 years and most (57.1%) were premenopausal. Clinical stages of patients ranged between T2N1 and T3N2. The pathological complete response (pCR) rate was 14.9 % (N=9). Two (5.7%) patients who were ER positive prior to treatment showed ER negativity after treatment. In 7 (21.1%) patients PR became negative after neoadjuvant chemotherapy and in 3 (9.0%) patients PR became positive. Changes in ER and PR receptors were not statistically significant (ER p=0.500 and PR p=0.549, respectively), whereas in 2 (5. 8%) patients hormonal status changed significantly when compared to initial biopsies (p=0.003).*

In addition, median value for PR intensity decreased from 20 to 10% (p=0.003) and Ki-67 values decreased from 10 to 1% (p<0.001) following neoadjuvant therapy. Six (17%) patients exhibited some changes in HER 2 staining. HER 2 expression became 2+ in 3 patients who were HER 2 negative prior to treatment, and HER 2 expression became negative in two patients with HER 2 1+ and 2+ prior to treatment following neoadjuvant chemotherapy.

Conclusion: *The biological markers ER, PR, HER 2 and Ki-67 index demonstrated differences after neoadjuvant treatment in breast cancer patients. These changes may affect the treatment decision.*

Key words: *breast cancer, ER, PR, HER 2, Ki -67, neoadjuvant chemotherapy*

Introduction

Treatment options in breast cancer depend on several factors, such as tumor grade, disease stage, molecular properties of the tumor and patient characteristics. These factors are all taken into account in order to choose the most proper treatment modality for each patient. Neoadjuvant chemotherapy is one of these treatment modalities, once used only in locally advanced breast cancer; nowadays it is more frequently used as an initial treatment option for operable patients [1].

It is very well known that chemotherapeutic agents cause some fundamental changes in the cell, leading to cell death [2-5]. However, very few studies investigated whether molecular changes beyond the lethal process are initiated with chemotherapy in cancer cells. While deciding

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the next adjuvant treatment, it is critical to know whether a neoadjuvant chemotherapy regimen changes some of the tumor biologic markers or causes selection of a tumor fraction which is biologically different from the chemotherapy-naïve tumor.

In this study, we aimed to evaluate the changes of breast cancer biologic markers ER, PR, HER 2 and Ki-67 index in locally advanced breast cancer patients after neoadjuvant chemotherapy.

Methods

From December 2005 to December 2010, patients with breast cancer and clinical stage II or III planned for surgery were retrospectively analyzed. Patients with early stage or metastatic breast cancer were excluded. Sixty-three patients whose initial histologic diagnosis was performed by core biopsies were included into the study. The patients received dose-dense therapy consisting of 4 cycles of 600 mg/m^2 cyclophosphamide, 60 mg/m^2 doxorubicin every 15 days followed by 4 cycles of paclitaxel 175 mg/m^2 every 15 days, after which they underwent surgery within two weeks after the last chemotherapy cycle. Pre and postoperative expression of ER, PR, HER 2 and Ki-67 of the tumor tissue specimens were evaluated and compared. Nuclear staining in >5% of tumor cells was accepted as positive for ER and PR. Ki-67 was calculated by counting the cells with positive Ki-67 nuclear staining among 1000 invasive tumor cells. Evaluation of HER 2 status was performed immunohistochemically according to the ASCO/CAP guidelines, as 4 graded system (0-3+).Specimens with 2+ by this method were further examined with fluorescence *in situ* hybridization (FISH) method. To determine the HER 2 positivity rate using FISH, the FDA-approved cut-off ratio (HER 2 signals/chromosome 17 signals) of 2.0 was used. All specimens were reviewed by two pathologists at the same time.

Statistics

Normally distributed continuous variables were demonstrated as means ± standard deviations and skew-distributed continuous variables as medians with range. Categorical variables were presented as percentages. For comparisons between groups, the Mann-Whitney U test was used for skew-distributed continuous variables and the McNemar test was used for dependent categorical variables. The statistical Package for Social Sciences (SPSS) 17,0 was used. Statistical significance was assumed at <0.05.

Results

The mean patient age was 49.2 ±10.7 years. Thirty-six (57.1 %) of them were premenopausal, 23 (36.5 %) postmenopausal and 4 (6.3 %) perimenopausal. Histologic classification of the tumors was as follows: 49 (77.8 %) patients had infiltrative ductal carcinoma, 9 (14.3 %) patients had mixed type carcinoma (infiltrative ductal carcinoma +lobular carcinoma), and 5 (8.0%) patients had other subtypes.

Clinical staging was assessed clinically and radiologically.Stages ranged between T2N1 and T3N2. Most patients (87.3%) underwent radical mastectomy. Three of the tumors were grade 1 (4.8%), 28 (44.4%) grade 2 and 24 (38.1%) grade 3. In 8 (12.7%) patients tumor grade was undetermined.

The overall clinical response rate (complete plus partial response) obtained with neoadjuvant therapy was 88.8 % (N=56). Forty six percent (N=29) of the patients had a complete clinical response and 42.8% (n=27) had a partial clinical response. In 6 (9.5%) patients disease remained stable after neoadjuvant therapy. The pathological complete response rate was 14.9 % (N=9). Clinicopathological characteristics and response to neoadjuvant chemotherapy are shown in Table 1.

Due to technical reasons it was not possible to study the biologic markers in the residual tumor after neoadjuvant therapy in all patients with incomplete pathological response. Qualitative changes in ER and PR receptor before and after therapy are shown in detail in Table 2.

Of the 35 patients whose ER change was evaluated, 33 (94.2%) conserved the same ER status. Two (5.7%) patients who were ER positive prior to treatment showed ER negativity after treatment. Thirty-three patients were assessed for their PR status and 23 (69.2%) of them conserved the same PR status. In 7(21.1%) patients PR became negative after neoadjuvant chemotherapy and in 3 (9.0%) patients PR became positive after neoadjuvant chemotherapy. Changes in ER and PR receptors were not statistically significant (ER p=0.500 and PR p=0.549, respectively).

How the changes in hormone receptors affected the hormonal status of the patients is shown in Table 3. Hormonal status remained unchanged in 32 (94.1%) patients although the hormone receptors were affected by the treatment. While in 2 (5.8%) patients the hormonal status changed when compared to initial biopsies (one of them who was initially hormone-negative converted to hormone-positive and the other patient who was initially hormone-positive converted to hormone-negative). This difference between the hormonal status of the patients pre and post treatment was statisti-

Table 1. Patient and disease characteristics and response to neoadjuvant therapy

Characteristics	*N (%)*
Age, years (mean±SD)	49.2 ±10.7
Menopausal status	
Premenopausal	40 (63.4)
Postmenopausal	23 (36.5)
Comorbid diseases	
No	37 (58.7)
Hypertension	12 (19.4)
Diabetes mellitus	5 (7.9)
Hyperlipidemia + coronary artery disease	8 (12.6)
Thyroid disease	3 (4.7)
Histologic type	
Infiltrative ductal carcinoma	49 (77.8)
Mixed (infiltrative ductal+lobular)	9 (14.3)
Metaplastic carcinoma	3 (4.8)
Others	2 (3.2)
Tumor grade in initial biopsy	
1	3 (4.8)
2	28 (44.4)
3	24 (38.1)
Could not be assessed	8 (12.7)
Response to neoadjuvant treatment	
pCR	9 (14.9)
cCR	56 (88.8)
PR	27 (42.8)
SD	6 (9.5)
PD	1 (1.5)

Table 2. Assessment of qualitative changes in ER and PR after neoadjuvant chemotherapy

	Initial tumor	*Residual tumor*	*N (%)*
ER (N=35)	Positive	Positive	29 (82.8)
	Positive	Negative	2 (5.7)
	Negative	Positive	-
	Negative	Negative	4 (11.4)
PR (N=33)	Positive	Positive	17 (51.1)
	Positive	Negative	7 (21.1)
	Negative	Positive	3 (9.0)
	Negative	Negative	6 (18.1)

cally significant (p=0.003).

Besides qualitative changes, the quantitative changes in hormone receptors were also evaluated. The change in the intensity of hormone receptors before and after neoadjuvant therapy is shown in Table 4. The median ER intensity in the core biopsy material prior to treatment was 50 (range 0-90), and after treatment it was 70 (range 0-90) (p=0.75). The median PR intensity prior to treatment was 20 (range 0-90), and after treatment it was 10 (range 0- 90) (p=0.003). Ki-67 values decreased significantly from 10 to 1% following neoadjuvant therapy (p<0.001).

Thirty-six patients were evaluated for possible change in the HER 2 status of the tumor. In 30 (83%) of them HER 2 expression remained unchanged, whereas 6 (17%) patients exhibited changes to some degree. HER 2 expression became 2+ in 3 patients who had negative HER 2 prior to treatment, and it became negative in 2 patients who had 1+ and 2+ following neoadjuvant therapy. When the specimens which converted to 2+ after therapy were further evaluated by FISH method, no gene amplification was found. HER 2 expression changes are shown in Table 5.

We did not find statistically significant difference between histological subgroups regarding ER or PR change (p=0.19). In addition, we observed a higher rate of complete clinical response in women who were postmenopausal and had T2 or lower tumor grade at the time of diagnosis (p=0.04 and p<0.001)

Discussion

The fact that chemotherapeutic agents cause some changes on some components of the tumor cells is known since the 1960s. First, Waller demonstrated changes such as enlargement of the nucleus, swelling of the cytoplasm, vacuolization in the cytoplasm/nucleus in tumor cells following systemic administration of busulphan [2]. Since changes in the molecular properties of cancer cell may affect the tumor behavior and therefore the

Table 3. Changes in hormonal status after neoadjuvant chemotherapy

	After treatment		
Before treatment	*Hormone negative (ER and PR negative) N (%)*	*Hormone positive (ER or PR positive) N (%)*	*p-value*
Hormone negative (ER and PR negative)	3 (8.8)	1 (2.9)	0.003
Hormone positive (ER or PR positive)	1 (2.9)	29 (85.3)	

treatment plan to be followed, studies investigating how the chemotherapeutic agents affect tumor grade, receptor properties of tumor cells and tumor proliferation rate have been increasing in number recently. In this study we examined the qualitative and quantitative changes in ER, PR, HER 2 and Ki-67 in breast cancer patients receiving neoadjuvant dose-dense chemotherapy with doxorubicin and cyclophosphamide, followed by paclitaxel.

Several studies looked at hormone receptor changes with neoadjuvant chemotherapy in tumor cells. Taucher et al. studied the effect of neoadjuvant therapy in a group of 214 patients and reported that 14% of the tumors which were ER positive and 51% of the tumors which were PR positive initially, became hormone receptor negative and both changes were statistically significant (p=0.02 for ER and p=0.0005 for PR) [6]. In a study by Makris et al. 11 patients showed a statistically significant change in ER status (p=0.04) and 15 patients showed a change in PR status which was not statistically significant [7]. There are also some other studies which demonstrated that neoadjuvant therapy results in changes in hormone receptor status in breast cancer [8,9]. On the other hand, some investigators support the idea that, possible changes caused by neoadjuvant therapy in hormone receptor status of the tumor do not show significant importance [10-15]. In our study 2 (5.7 %) patients who were ER positive before neoadjuvant treatment became ER negative and this change was statistically significant (p<0.001). Ten patients (30.1%) showed a change in PR status; 7 (21.1%) of them converted from PR positive to negative and 3 (9.0%) patients converted from PR negative to positive. However, these changes were not statistically significant (p=0.160).

Most of the studies about Ki-67 reported a decrease in this index after neoadjuvant therapy. Studies by Makris et al. and Yin et al. showed a statistically significant decrease in Ki-67 proliferation index following neoadjuvant chemotherapy (p=0.001 and p=0.01, respectively) [7,16]. Bottini et al. and Pohl et al. reported similar results confirming decrease in Ki-67 index [10,18]. In a study by Koda et al., Ki-67 significantly decreased in the primary breast tumor when compared to pre-treatment values, although no change was observed in metastatic lymph nodes with neoadjuvant chemotherapy [18]. In concert with other studies in the literature, our study showed a significant decrease in Ki-67 with neoadjuvant chemotherapy from a median 10% prior to therapy to 1% after therapy (p<0.001). This observation may be related with the conversion of the whole tumor cell population to a less proliferative state or may be the selection of the less proliferative cells by means of neoadjuvant treatment. But in either case the results of our study confirm the studies which attribute the decrease of Ki-67 to neoadjuvant chemotherapy.

Table 4. Percent changes in median values of hormone receptor intensity and Ki-67 index before and after neoadjuvant therapy

Hormone receptor intensity Median % (range)	*Initial tumor*	*Residual tumor*	*p-value*
ER	50 (0-90)	70 (0-90)	0.753
PR	20 (0-90)	10 (0-90)	0.003
Ki-67 Median % (range)	10 (1-60)	1 (1-1)	<0.001

Table 5. Changes in HER2 expression assessed immunohistochemically

Changes in HER 2 expression	*N (%)*	*p-value*
Increase	3 (8.4)	< 0.001
Decrease	3 (8.4)	
No change	36 (83)	

Another important issue in the treatment of breast cancer is the changes in HER 2 status of the tumor. The variations of this biological marker in primary tumor and its synchronous/metachronous metastasis and changes in HER 2 status after neoadjuvant treatment were assessed in several studies [19,20]. Studies examining the effect of neoadjuvant chemotherapy on HER 2 expression report conflicting results. Adams et al. reported increased expression of HER 2 after neoadjuvant chemotherapy in breast cancer patients in whom the hormone receptor status remained unchanged [13].

Taucher et al. [6] evaluated HER 2 changes with immunohistochemical methods in addition to hormone receptor changes in their study. They showed that HER 2 positivity changed after neoadjuvant chemotherapy but the results were not statistically significant. They also reported that confirmation of the results with FISH method revealed that the difference was much smaller compared with immunohistochemistry. Another study by Burcombe et al. showed a change in HER 2 expression in 9 of 118 patients with neoadjuvant chemotherapy (from 3+ to 2+ in 3 patients and from 2+ to 3+ in 5 patients) and the authors suggested reevaluation of HER 2 in the residual tumor in patients whose initial HER 2 studies

showed mild to high positivity after neoadjuvant therapy [21]. In addition, Neubauer et al. reported 13% of the tumors switched from HER 2 positive to negative after neoadjuvant therapy [22]. On the other hand, several studies report no change in HER 2 expression with neoadjuvant therapy [23-25].

In our study we observed that HER 2 expression remained unchanged in 30 (83%) of the 36 patients, while some degree of change occurred in 6 (16.6%) patients. In 3 patients HER 2 converted to 2+ positive from negative and in one patient HER 2 expression converted to negative from 1 +. However, when we reevaluated this particular case with FISH method we found that none of the 3 samples showed HER 2 amplification. So our results support the studies which report no change in HER2 expression with neoadjuvant treatment.

Our study has some limitations. The number of the cases studied was less than expected due to technical problems, such as problems in conservation of the tissue samples and technical problems during specimen staining procedures. Immunohistochemical methods used in the evaluation of HER 2 and hormone receptors may be affected from different factors. For example, improper tissue sampling and conservation under inappropriate conditions until fixation, insufficient amount of tissue specimen or sampling made from an area which does not represent the heterogeneity within the tumor may all affect the results. Direct effects of the chemotherapeutic agents themselves on the immunohistochemical staining and factors related to the examining pathologist are additional important factors. In our study the histological evaluation was made by consensus of two different pathologists who were specialized in this area.

The biological effects of chemotherapeutic agents on cancer cell other than cell death are issues that should be thoroughly investigated. Understanding the chemotherapy-induced biological conversion in the tumor cell behavior is strategically important in planning adjuvant therapy and for disease follow up. Studies on different cancer types and on larger populations may help understand the effects of chemotherapeutic agents on tumor biology.

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