Locally advanced breast carcinoma treated with neoadjuvant chemotherapy: are the changes in serum levels of YKL-40, MMP-2 and MMP-9 correlated with tumor response?

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Serum levels of YKL-40, MMP-2 and MMP-9 in 27 patients with locally advanced breast carcinoma received neoadjuvant chemotherapy were measured. All patients underwent neoadjuvant chemotherapy named as FAC protocol (5-Fluorouracil, Doxorubicin and Cyclophosphamide) with 21 days interval. There was 26,7% decrease in mean serum YKL-40 levels (from 146,4 μg/ml to 107,3 μg/ml) in clinically responsive group. This level was almost unchanged in non-responsive group (P>0, 05). There was 42, 1% decrease in mean serum YKL-40 levels (from 173,1 μg/ml to 98, 8 μg/ml) in pathologically responsive group. This decrease was more dramatic in patients with complete pathological response (70, 2%). However, this level was slightly increased in non-responsive group. Changes in serum levels of MMP-2 and MMP-9 were not found to be associated with tumor response. Serum measurement of YKL-40 can be a helpful tool to predict pathological tumor response in breast cancer patients with neoadjuvant chemotherapy but not MMP-2 and MMP-9.

Key words: Locally advanced, breast cancer, neoadjuvant, YKL-40, MMP-2, MMP-9

Systemic treatment before surgery as called neoadjuvant chemotherapy is widely accepted approach in patient with locally advanced breast cancer. It is usually difficult to quantify tumor response using clinical examination of breast or imaging methods only. Ability to access to the primary tumor affords the opportunity in vivo sensitivity testing and for evaluating the mechanism of action of a therapy and molecular changes that may reflect tumor response [1].

YKL-40, a member of the mammalian family 18 glycosylhydrolayses, is secreted by cancer cells and macrophages [2, 3, 4] and expressed selectively by murine mammary tumors initiated by neu/ras oncogenes [3]. It has been suggested that YKL-40 may play a role in the proliferation and differentiation of malignant cells, protects the cancer cells from apoptosis, stimulates angiogenesis, has an effect on extracellular tissue remodeling, and stimulates fibroblasts [5]. It has been shown that YKL-40 is expressed by several types of carcinoma including colon, lung, kidney, ovarian, prostate, uterine, glioblastoma and breast [6-9]. Moreover, several studies have shown that elevated serum concentrations of YKL-40 in patients with breast, colorectal, ovarian, kidney, prostate, small cell lung cancer and malignant melanoma was an independent prognostic variable of short recurrence free interval and short overall survival [5, 10-18].

Matrix metalloproteinases (MMPs) are a large family of proteolytic enzymes of extracellular matrix [19]. MMPs play important role in extracellular matrix degradation, tumor cell invasion, metastasis and angiogenesis [20-22]. The gelatinases or collagenases type IV, MMP-2 (Gelatinase A, 72 kDa) and MMP-9 (Gelatinase B, 92 kDa) are capable of degrading components of the basement membrane, particularly collagen type IV, the first vital barrier breached by tumor cells. Prognostic significance of serum MMP-2 and MMP-9 levels has been reported in some studies including breast carcinoma [19, 23-25].

In this study, we measured serum levels of YKL-40, MMP-2 and MMP-9 in patients with locally advanced breast carcinoma and evaluated whether the changes in these levels predict the response to neoadjuvant chemotherapy.

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Table1. Serum levels of YKL-40, MMP-2 and MMP-9 before and after chemotherapy according to clinical tumor response

Response	n(%)	Median age (range)(yrs)	YKL-40 (mean±SD) (μg/ml)		MMP-2 (mean±SD) (ng/ml)		MMP-9 (mean±SD) (ng/ml)	
			Before	After	Before	After	Before	After
SD	6 (22,3)	39,5 (35-44)	160,4±161,4	155,2±188,6	636,9±236,5	628,8±391,1	148,7±70,9	169,9±54,3
CR	2 (7,4)	42,5 (38-47)	135,2±113,8	104,0±75,1	613,8±502,1	629,6±33,2	155,8±14,7	219,2±91,9
PR	19 (70,3)	53,0 (27-65)	147,6±217,5	107,7±75,6	547,4±337,9	600,4±370,5	146,9±35,1	148,5±66,6
CR+PR	21 (77,7)	53 (27-65)	146,4±207,9	107,3±73,7	553,7±340,3	603,2±351,7	147,8±33,6	155,2±69,7
Total	27 (100)	45 (27-65)	149,5±195,7	118,8±106,9	572,2±317,9	608,9±353,1	148,0±42,8	158,5±65,9

p>0, 05, CR+PR Responsive group, SD Non-responsive group

Material and Methods

Twenty-seven locally-advanced breast carcinoma patients were included in the study. Fifteen had stage IIIA and 12 stage IIIB disease. Sixteen (59, 2%) patients had hormone receptor positive disease. All patients underwent neoadjuvant chemotherapy named as FAC protocol (5-Fluorouracil 600 mg/m2 1. day, Doxorubicin 60 mg/m2 1. day and Cyclophosphamide 600 mg/m2 1. day) with 21 days interval. Before and 3 cycles after chemotherapy, all patients were evaluated by physical examination and ultrasonography /mammography of breast. At the time of study, HER-2 receptor was not routinely analyzed in all breast cancer patients and trastuzumab treatment had not been approved yet. Therefore, we don't know the HER-2 status of the patients. Response was judged by standard WHO criteria [26]. Responders were defined as complete response (CR, disappearance of assessable disease) or partial response (PR, reduction of more than 50% of the lesion of the two largest tumor diameter). Stable disease (SD) meant less than 25% increases in tumor size. Progressive disease (PD) was defined by an increase of more than 25 % in tumor size. Pathological complete response meant no tumor in pathological examination of resected breast and axillary lymph nodes. Blood samples from patients were taken before and 3 cycles after the chemotherapy into dry tubes, allowed clotting at 4 C for maximal 2 hrs and the sera separated from cellular elements by centrifugation at 1600 g at 4 C for 10 min. The sera were stored at – 80 C until analysis.

Serum YKL-40 was determined by ELISA (Quidel Corporation, Santa Clara, CA, USA). The YKL-40 assay is a sandwich enzyme immunoassay in a microtiter stripwell format. The Fab fragment of monoclonal anti-YKL-40 antibody conjugated to biotin binds to streptavidin on the strip and captures YKL-40 in a standard control and sample. A polyclonal anti-YKL-40 antibody conjugated to alkaline phosphatase binds to the captured YKL-40. Bound enzyme activity is detected with p-nitrophenyl phosphate as substrate. The sensitivity of the ELISA was 20 ng/ml. The intra-assay and inter-assay coefficient of variation were 6,6 % and 6,8 %, respectively.

Serum MMP-2 and MMP-9 concentrations were determined by using human MMP-2 and MMP-9 enzyme-linked immunosorbent assays (ELISA) (Ray Bio tech, Inc., according to manufacturers instructions, as previously described [27]. The intra and inter-assay coefficients of variation for both tests were <10% and <12% respectively. The minimum detectable dose of MMP-9 was less than 10 pg/ml and the minimum detectable dose of MMP-2 was less than 140 pg/ml.

Statistical analysis

Mann-Whitney U Test, Wilcoxon Signed Ranks Test and Pearson's Correlation Analysis were used for statistical analysis. P values less than 0, 5 were accepted as significant.

Results

After 3 cycles of FAC chemotherapy, 19 (70, 3%) patients had PR, 2 had CR (7, 4%) and 6 had SD (22, 3%) with clinical evaluation including physical examination and ultrasonography /mammography of breast. After 3 cycles of chemotherapy, while 3 patients who had stable disease received a new regimen including taxans, the other 24 patients underwent immediate surgery (modified radical mastectomy and axillary lymph node dissection) without any additional chemotherapy cycles. There was some inconsistency in pathological examination of tumor response compared to the results of clinical evaluation. Three patients who initially thought to have PR with clinical evaluation had stable disease and 2 had complete response in pathological examination. One patient who initially thought to have CR with clinical evaluation had PR in pathological examination. Among 6 patients who initially thought to have SD with clinical evaluation, 2 had PR in pathological examination.

Serum levels of YKL-40, MMP-2 and MMP-9 before and after chemotherapy according to clinical tumor response and pathological response are shown in Table 1 and Table 2, respectively. There was no decrease in serum MMP2 and MMP-9 levels after chemotherapy both in responsive and non-responsive group according to clinical evaluation (p>0, 05). Although there was an increase in serum MMP-2 levels in responsive group according to pathological evaluation, these levels were found to be decreased in non-responsive group (p>0,05). There was no decrease in serum MMP-9 levels ac-

Table 2. Serum levels of YKL-40, MMP-2 and MMP-9 before and after chemotherapy according to pathological tumor response

Response	n(%)	Median age (range)(yrs)	YKL-40 (mean±SD) (μg/ml)		MMP-2 (mean±SD) (ng/ml)		MMP-9 (mean±SD) (ng/ml)	
	Before	After	Before	After	Before	After		
SD	5 (20,8)	44,0 (38-58)	109,7±32,8	124,5±105,4	620,9±270,9	413,6±196,2	181,6±26,8	187,1±53,3
CR	3(12,5)	40,0 (39-47)	185,7±34,1	55,4±4,4	358,1±223,8	459,1±245,1	163,2±20,6	133,2±29,0
PR	16 (66,7)	53,5 (27-65)	170,7±251,6	106,9±61,9	583,9±356,0	717,7±369,1	143,5±42,9	155,8±76,8
CR+PR	19 (79,2)	47,0 (27-65)	173,1±230,0	98,8±59,7	548,2±344,0	676,8±360,0	146,6±40,5	152,2±71,3
Total	24 (100)	46,0 (27-65)	159,9±205,6	104,1±69,6	563,4±326,0	622,0±346,4	153,9±40,2	159,5±68,4

* Three patients who received additional cycles were excluded from the analysis
p>0, 05, CR+PR Responsive group, SD Non-responsive group

Table 3. Changes in serum levels of YKL-40, MMP-2 and MMP-9 with neoadjuvant chemotherapy according to clinical tumor response (difference between the value after chemotherapy and the value before chemotherapy)

Response	n (%)	YKL-40 (μg/ml)	MMP-2 (ng/ml)	MMP-9 (ng/ml)
(–)	6 (22,3)	-5,2±279,8	-8,11±425,9	21,1±44,7
(+)	21 (77,7)	-39,0±229,5	49,4±445,5	7,4±71,6

p>0, 05

Table 4. Changes in serum levels of YKL-40, MMP-2 and MMP-9 with neoadjuvant chemotherapy according to pathological tumor response (difference between the value after chemotherapy and the value before chemotherapy)

Response	n (%)	YKL-40 (μg/ml)	MMP-2 (ng/ml)	MMP-9 (ng/ml)
(–)	5 (20,8)	14,8±120,0	-207,0±233,0	5,5±34,8
(+)	19 (79,2)	-74,3±244,2	128,6±464,8	5,5±75,1

* Three patients who received additional cycles were excluded from the analysis
p>0, 05

cording to pathological evaluation. Serum YKL-40 levels were found to be decreased after chemotherapy particularly in responsive group according to clinical evaluation. While this decrease for YKL-40 became clearer in responsive group according to pathological evaluation, there was no decrease in non-responsive group. However, these results were not statistically significant (p>0, 05) (Table 1, 2, 3, 4).

Initial and post-chemotherapy levels of serum YKL-40, MMP-2 and MMP-9 and their changes during and after chemotherapy were not associated with disease free and overall survival of patients (p>0, 05).

Discussion

Neoadjuvant chemotherapy is a standard treatment of locally advanced breast cancer. Since clinical response of tumor with neoadjuvant chemotherapy does not reliably reflect pathological finding of surgical specimen [28], there may be some difficulties for clinicians in deciding to continue with same chemotherapy or change it with other agents or go to immediate surgery after 2 or 3 cycles of initial neoadjuvant chemotherapy regimens. Chemotherapeutic agents may exert their effect by decreasing proliferation, increasing apoptosis or both [1]. While proliferation and apoptosis are relatively global assessments of response, chemotherapeutic agents may target specific individual molecules that regulate the process [1].Therefore, some molecular markers which may help to quantify tumor response can be useful in clinical practice of physicans.

YKL-40 is secreted by cancer cells and macrophages [2, 4]. It has been shown that serum YKL-40 levels were associated with prognosis of patients with breast, colorectal, ovarian, kidney, prostate, small cell lung cancer and malignant melanoma [5, 10-18].

MMPs play imported roles in extracellular matrix degradation, tumor cell invasion, metastasis and angiogenesis [20-22]. Prognostic significance of serum MMP-2 and MMP-9 levels has been reported in patients with breast cancer [19, 23-25].

In our study, we measured serum YKL-40, MMP-2 and MMP-9 levels before and after neoadjuvant chemotherapy of locally advanced breast carcinoma patients. When we grouped the patients as clinically responsive (CR+PR) and non responsive group (SD) with neoadjuvant chemotherapy, there was 26,7% decrease in mean serum YKL-40 levels (from 146,4 μg/ml to 107,3 μg/ml) for clinically responsive group. This decrease was 22, 9% in patients with clinically CR patients. However, this level was almost unchanged in non-responsive group (Table 1).

In many studies, a discrepancy was noted between clinical CR and pathological CR [29-31]. Similarly, in our study, 2 patients who were clinically evaluated as PR had no tumor in their pathological examination. Moreover, one patient who had clinically CR had residual tumor in surgical specimen. This can be attributable to overestimation of the residual tumor from chemotherapy-induced fibrosis or difficulty in detecting microscopic residual tumor by the classical evalua-

tion methods we used. According to pathological findings, we re-grouped the patients as seen in table 2. Three patients did not include in this analysis, because they did not undergo surgery after 3 cycles of initial chemotherapy. In this analysis, there was 42, 1% decrease in mean serum YKL-40 levels (from 173,1 μg/ml to 98, 8 μg/ml) for pathologically responsive group (CR+PR). This decrease was more dramatic in patients with pathological CR (70, 2%). However, this level was slightly increased in non-responsive group (SD).

Although none of these differences is statistically significant most probably due to small number of patients, we can consider that changes in serum YKL-40 levels may be helpful to estimate pathological response after chemotherapy. Changes in serum levels of MMP-2 and MMP-9 were not found to be associated with tumor response.

In conclusion, serum YKL-40 measurement seems to be a good candidate to reflect pathological tumor response in breast cancer patients with neoadjuvant chemotherapy but not MMP-2 and MMP-9. This is just a small pilot study but further studies with more patients would clarify this suggestion.

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