Predictive value of relative changes in serum total sialic acid level for response to neoadjuvant chemotherapy in patients with locally advanced breast carcinoma

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The aim of this study was to determine whether relative changes in total serum sialic acid (TSA) levels are associated with response to neoadjuvant chemotherapy in locally advanced breast carcinoma (LABC) patients. Forty-seven patients with stage III-B breast carcinoma and 20 healthy subjects (controls) were included to the study. TSA levels were determined in serum from patients at baseline and after completion of preoperative chemotherapy. Pathological responses to chemotherapy were determined on specimens of modified radical mastectomy underwent in responders. Association between the relative changes in serum TSA levels and the pathological response to chemotherapy was investigated. The baseline mean serum TSA level of LABC patients was 88.6±0.6 mg/dl and 66.9±0.7 mg/dl for the control group ($p<0.0001$). After 3 cycles chemotherapy, the serum levels of TSA were markedly decreased with pathological partial response (pPR) (73.8±1.0 mg/dl) and complete response (pCR) (68.1±1.9 mg/dl) compared to baseline values ($p<0.05$). In 8 non-responders, mean TSA value was 88.9±1.1 mg/dl ($p=0.9$ for pretreatment vs posttreatment TSA levels). Of 39 responders, 6 had pathological complete response (pCR) and remaining had pathological partial response (pPR). TSA levels derived from patients with pCR and from those with pPR were 68.1±1.9 mg/dl and 73.8±1.0 mg/dl, respectively ($p=0.03$). While TSA levels from pCR were not different from those of controls ($p=0.4$), there was a significant difference between TSA levels from pPR and from controls ($p<0.0001$). A significant correlation was demonstrated between the relative changes in TSA levels and pathological response ($p<0.0001$, coefficient of correlation [r_s]=0.81). The ROC analysis showed that the discriminating ability was satisfactory and relative decrease by more than 21% in TSA levels indicated a pCR with the sensitivity by 83%, specificity by 76%. In conclusion, there is a significant correlation between the relative changes in TSA levels by chemotherapy and clinical/pathological response to neoadjuvant chemotherapy in LABC patients.

Key words: sialic acid, response, neoadjuvant chemotherapy, breast carcinoma

In patients with locally advanced breast carcinoma, the most typical approach currently is to use a neoadjuvant chemotherapy regimen [1–3]. Neoadjuvant chemotherapy allows for individual in vivo assessment of tumor response to chemotherapy, and tumor downstaging with neoadjuvant chemotherapy can convert inoperable disease to operable disease [2]. Clinical response to neoadjuvant chemotherapy determined by physical examination and supplementary investigations such as mammography and/or ultrasonography, does not always correlate with pathologic response [1, 3]. Several studies have showed that the achievement of surgical specimens without residual tumor was the most significant factor in predicting a prolonged disease-free survival and overall survival [3]. At present, very limited information is available regarding determining of the pathological response in these patients before surgery.

Sialic acids present as components of soluble and cell surface glycoconjugates in animal cells and tissues, appear to be involved in the regulation of cell surface phenomena and thus in malignant transformation [4, 5]. Malignant transformation leads to elevated plasma sialic acid concentration. Total sialic acid (TSA) levels higher than those for controls have been reported in serum from patients with breast carcinoma [6]. It may be expected that these high concentrations of TSA in patients with breast carcinoma will fall if tumor has disappeared after treatment of carcinoma. To date, the literature contains

no study of a possible association between relative changes induced by chemotherapy in TSA levels and pathological response to neoadjuvant chemotherapy in LABC.

This prospective study was undertaken to determine, whether relative changes in serum TSA levels after neoadjuvant chemotherapy correlate with the extent of tumor response to chemotherapy in stage III-B breast carcinoma patients treated from 2001 to 2004 at Ankara Oncology Hospital.

Material and methods

Patient selection. The eligibility criteria of this study were as follows: pathological diagnosis of infiltrative ductal carcinoma by incisional biopsy, having T_{4b} tumor, no inflammatory tumors, no metastatic spread, no prior specific treatment and planning neoadjuvant chemotherapy with FAC regimen (5-Fluorouracil 600 mg/m^2 i.v., doxorubicine 60 mg/m^2 i.v., and cyclophosphamide 600 mg/m^2 i.v., every 21 days).

Staging before neoadjuvant chemotherapy was carried out according to AJCC system [7]. Initial staging included complete and detailed clinical examination, bilateral mammography, breast ultrasonography and pathological diagnosis for primary tumor. Every patient was proven to be devoid of metastases by chest x-ray, liver ultrasonography and bone scintigraphy. Biological assessment comprised blood cell count, electrolytes and serum creatinine, alcaline phosphatase, gamma glutamyl transferase and tumor marker (CA 15.3). The response to neoadjuvant chemotherapy was evaluated by physical examination and by the same methods mentioned above, according to International Union Against Cancer criteria [8]. The disappearance of T_{4b} tumor features and also total disappearance of tumor mass (clinical complete response) or ≥50% reduction in tumor mass (clinical partial response) was determined as the clinical objective response. A modified radical mastectomy was performed in the responders. On the data obtained from these materials, pathological complete response (pCR) was defined as a disappearance of all invasive disease from the breast and lymph nodes. Invasive tumor measuring any size in the breast or any positive lymph node in the axilla regardless of the residual disease in the breast was considered as a pathological partial response (pPR). The correlation between relative changes in TSA levels by chemotherapy and, patients' response status and clinico-pathological features was evaluated.

Sample preparations. Whole blood samples, after informed consent, were obtained from 47 consecutive stage III-B non-inflammatory breast carcinoma patients before starting the neoadjuvant chemotherapy as baseline and from 20 healthy subjects as controls. All subjects had no other disease potentially compromised to the TSA level, such as recent trauma, infection, etc, and no abnormalities in rutin biochemistry. Blood samples were re-obtained in all patients after three cycles of chemotherapy and also in responders incorporated in the study after one month of surgery.

The drawn blood was allowed to coagulate at room temperature and centrifuged at 2000 rpm for 15 minutes. The resulting sera were removed and stored frozen at –20 °C until the analysis was performed.

Determination of total sialic acid. TSA levels were determined according to the thiobarbituric acid method [9]. TSA was quantified after hydrolysis of 0.1 ml sample in 0.9 ml 0.1 N H_2SO_4 at 80 °C for 1 hour. Each sample was analyzed in duplicate, and for each determination, spectrophotometric readings against blank were made at 549 and 532 nm to overcome interference from 2-deoxy-D-ribose. The concentration of TSA was calculated using simultaneous equations as described by Warren. The molecular extinction values for N-acetylneuraminic acid and 2-deoxy-D-ribose at 532 and 549 nm were calculated from calibration curves, obtained by using different dilutions of the commercial products under similar experimental conditions. Pretreatment, posttreatment, postoperative and control samples were assayed at the same time.

Statistical analysis. Statistical tests were performed using the SPSS 9.05® statistical software package for Windows (SPSS Inc., Chicago, IL, USA). This study's enpoints were to assess relative changes in TSA levels by chemotherapy and to determine the association between relative changes and pathological response to neoadjuvant chemotherapy. Relative changes in TSA levels were calculated as ratio of the values measured after chemotherapy and at baseline. Statistical comparisons were made by Student t-test for unpaired data, paired sample t-test for change in the TSA levels by chemotherapy, and two-way Kruskal-Wallis test with Wilcoxon rank-sum test as posthoc test for multiple comparison, where indicated. The Spearman rank correlation and logistic regression analysis were applied for evaluating the relationship between the relative changes in TSA levels and pathological response. A receiver operating characteristic (ROC) analysis was performed to investigate sensitivity and specificity of the relative changes in TSA levels on predicting pathological complete response. Mean values were given with their standard errors (sem). Statistical significance was assumed at $p<0.05$.

Results

Baseline and control TSA levels. The median ages were 55 (range: 30 to 75) years for 47 patients and 53 (range: 41 to 69) years for 20 controls ($p=0.5$). While mean serum TSA level in the control group was 66.9±0.7 mg/dl, mean serum TSA level in patients was 88.6±0.6 mg/dl (Fig. 1). The mean concentration of TSA was significantly higher in breast carcinoma patients than in control subjects ($p<0.0001$). A TSA range of control values were defined from 61.0 to 72.8 mg/dl, corresponding to mean ±2 S.D. Only 5% (n=2) of healthy subjects had TSA values higher than this cut-off level versus all LABC patients.

Posttreatment TSA levels. The treatment efficiency was

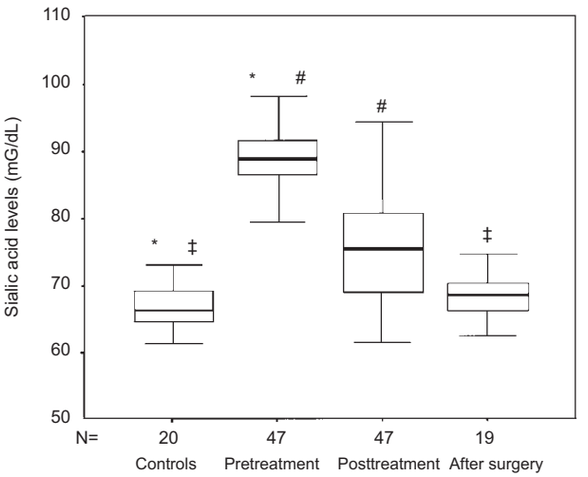

Figure 1. Box and whisker plot illustrating sialic acid levels in the patient groups. The figure shows median values (horizontal line), inter-quartile intervals (boxes) and 95% confidence intervals (whiskers). $^{*}p<0.00001$, $^{\#}p<0.0001$, $^{\ddagger}p=0.3$.

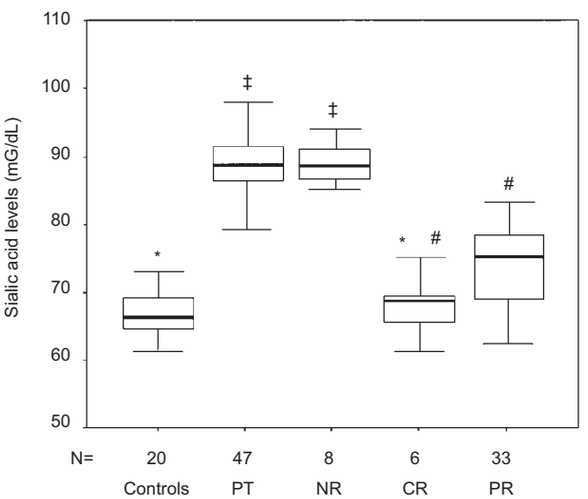

Figure 2. Sialic acid levels according to the types of response. PT – pretreatment, NR – no response, CR – complete response, PR – partial response. $^{*}p=0.4$, $^{\#}p=0.03$, $^{\ddagger}p=0.9$

evaluated after 3 cycles of chemotherapy. Objective response to chemotherapy was observed in 39 (83%) patients. After chemotherapy, the mean TSA level was 71.0±1.1 mg/dl in these responders. Comparison of TSA levels between pretreatment and posttreatment indicated statistically significant differences in these patients ($p<0.0001$), and the variation in responders' TSA indicated a decrease of mean 17%. TSA values according to the type of response are seen in Figure 2. There was statistically significant difference between mean TSA level and 88.9±1.1 mg/dl SA level in 8 non-responders ($p<0.0001$). Of the responders, 6 (15.4%) had pathological

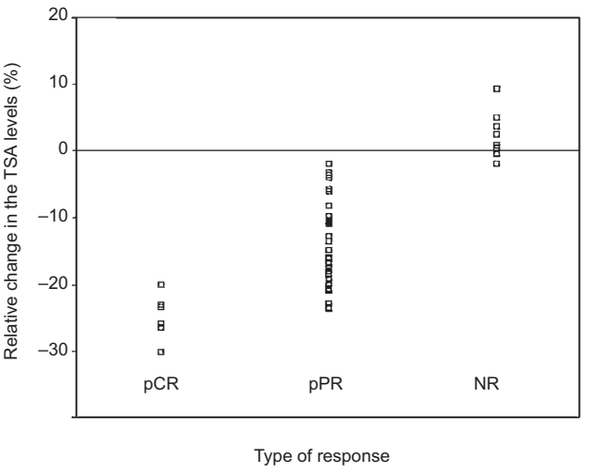

Figure 3. The relationship between the type of response and the relative change in the total sialic acid (TSA) concentration after 3 cycles of neoadjuvant chemotherapy. pCR – pathological complete response, pPR – pathological partial response, NR – no response.

complete response (pCR) and remaining had pathological partial response (pPR). The mean TSA values after chemotherapy were 68.1±1.9 mg/dl for the patients with pCR and 73.8±1.0 mg/dl for those with pPR (Fig. 2). We found statistically significant differences among response groups using the Kruskal-Wallis test; these groups were then compared in pairs applying the Wilcoxon rank-sum test. After chemotherapy, the difference between TSA levels derived from patients with pCR and from those with pPR was significant ($p=0.03$). Whereas no significant variation in mean TSA value was observed between pCR and controls ($p=0.4$), there was significant difference between TSA levels from pPR and from controls ($p<0.0001$). TSA values after surgery (67.9±0.7 mg/dl) in 19 of responders were not different from controls ($p=0.3$). Figure 3 shows the relative changes in the TSA levels according to the type of response.

Correlation between the relative changes in TSA levels by the type of response. Statistically significant correlation was demonstrated between the relative changes in TSA levels by chemotherapy, and the pathological response ($p<0.0001$, coefficient of correlation [r_s]=0.81). The logistic regression analysis showed that the relative changes in TSA levels were able to determine pathological response status ($p<0.0001$).

When the ROC analysis was applied on the data, area under the curves (AUC) was 0.81 ($p=0.01$). This analysis showed that the discriminating ability was satisfactory and the relative change by more than 21% in TSA levels due to neoadjuvant chemotherapy indicated pathologic complete response with the sensitivity by 83%, specificity by 76%. Based on this cut-off point (with negative predictivity by 96%), patients with low relative change had a risk of incomplete pathological response that was 9 times higher than their counterpart.

Discussion

Despite recent advances in screening mammography and increased public awareness of the importance of early detection, locally advanced breast carcinoma is diagnosed in 2% to 5% of all breast carcinomas [3]. The neoadjuvant chemotherapy regimens generally produce an overall clinical objective response rate up to 87% [10, 11], with response rates ranging from 3% to 46% (pathological complete response) and 30% to 93% (pathological partial response) [12], as in the present series. If the tumor does not respond to the initial chemotherapy regimen, substitution with other drugs may be beneficial [3]. On the other hand, the pathological tumor response has been reported to be a more powerful prognostic factor than clinical response in LABC [11, 13, 14]. Thus, maximal tumor shrinkage and its devitalization may represent a major goal of neoadjuvant chemotherapy. In an attempt to improve these response rates, and thus survival, different and more complicated approaches have been employed [11]. On the other hand, attempts are under way to determine whether there are molecular predictors of response to neoadjuvant chemotherapy before surgery [3].

In recent years, several studies on TSA levels in serum from patients with different malignant diseases including those with breast carcinoma, showing higher levels than those for controls, have been published [6, 15–22]. However, studies in which TSA levels were investigated as a response marker to neoadjuvant chemotherapy in locally advanced breast carcinoma, have not been reported. Sialic acids (the most common form being N-acetylneuraminic acid), present as components of soluble and cell surface glycoconjugates in animal cells and tissues, appear to be involved in the regulation of cell surface phenomena and thus in malignant transformation [15, 17, 23]. The quantity of glycoconjugates on membranes of neoplastic cells is higher than on membranes of normal cells. TSA includes a small amount of free sialic acid as well as glycoprotein and glycolipid bound sialic acid [22]. The increased serum TSA levels in patients with tumor have been explained by a spontaneous release of sialic acid containing cell surface glycoconjugates [6, 15, 19]. Some authors have suggested that increased serum SA concentrations in patients with cancer reflect an inflammation reaction to the tumor, leading to increased output of acute phase proteins from liver [17, 19]. We found significantly higher TSA levels in serum of breast carcinoma patients than in healthy subjects and this result was consistent with other reports [6]. The fact that 5% of healthy subjects had TSA levels above the normal range, 72.8 mg/dl in this study, could be explained by a lack of specificity. High levels of TSA are observed not only in patients with different types of cancer, but also in patients with cardiovascular diseases, rheumatoid arthritis and inflammatory reactions [16, 18]. However, when a high level of TSA is observed, malignant disease must be suspected [22].

In the present study, we examined interaction between the relative changes in TSA levels by neoadjuvant chemotherapy and response to a standart-dose, antracycline-based, preoperative chemotherapy regimen for locally advanced breast carcinoma. After 3 cycles chemotherapy, TSA levels were significantly lower than baseline levels and moreover, patients whose TSA levels after treatment were not different from these of controls experienced complete pathological response. TSA normalization might indicate that the tumor proliferation was stopped. There was no significant difference between basic values and levels from non-responders. TSA levels after surgery in responders were similar to those of controls. It is possible that with the decreased tumor volume, the sialoglycoconjugates content also decreases, and lower levels of SA release into the blood. Moreover, because of the lack of tumor mass, the expected inflammation reaction to the tumor that leads to increased output of acute phase proteins from liver will not occur. On the other hand, statistically significant correlation was demonstrated between relative changes in TSA levels by chemotherapy and pathological response. The logistic regression analysis showed that the relative changes in TSA were able to determine the pathological response status. Moreover, relative change of 21% in TSA levels after chemoterapy indicated pathological complete response. Therefore, TSA might provide useful information on the tumor response to treatment.

This study, although based on a very limited number of subjects, showed that relative change in TSA levels after chemotherapy in LABC patients had a higher sensitivity for differentiating between responders and non-responders, also between pathological complete responders and partial responders. TSA determinations before and after 3 cycles of chemotherapy, which are inexpensive, simple and rapid, may provide additional information to conventional methods for evaluation of response to neoadjuvant chemotherapy.

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