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Serum Levels of PAI-1 and TNF-α as a Predictor of Response to Anthracycline Based Chemotherapy in Locally Advanced Breast Carcinoma: A prospective study in capital city of India

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ABSTRACT

Background: Aim: To assess role of serum levels of PAI-1 and TNF alpha as predictor of response to anthracycline based neoadjuvant chemotherapy in locally advanced breast carcinoma. **Methods:** A prospective study was conducted in the twenty-five cases of locally advanced breast cancer. Clinico-demographic characteristics of patients, TNM stage of patients, TNF-α and PAI-1 levels before and after chemotherapy, histopathological characteristics, presence or absence of invasive carcinoma, tumor size, estrogen and progesterone receptor and HER-2-Neu status, histological Grade, and axillary lymph node status were studied. **Results:** The mean values of TNFα and PAI-1 levels were found to be significantly elevated with increase in histological grade of tumor. This correlation was statistically significant for TNFα. With TNFα as variable and response to neoadjuvant therapy as state, 30.7 pg/ml was derived as cut off value TNFα for to predict response to chemotherapy. (AUC = 1.000) with sensitivity and specificity of 100% and 93% respectively. Whereas, PAI-1 as variable and response to neoadjuvant therapy as state, 55.6 ng/ml was derived as cut off value PAI-1 for to predict response to chemotherapy. (AUC = 0.977) with sensitivity and specificity of 90% and 86.7% respectively. **Conclusion:** TNFα and PAI-1 were found to be independent predictive factor for response to chemotherapy, thus providing as a safe, easy, objective and convenient supplement to other known factors in assessing response to chemotherapy. Significant post-chemotherapy decrease in serum TNFα and PAI-1 levels may provide objective criteria to assess response to chemotherapy.

Keywords: Breast carcinoma, TNFα, PAI-1, Neoadjuvant chemotherapy, Anthracyclines.

INTRODUCTION

Breast cancer is the most common cancer in women and accounts for 29% of all cancers diagnosed each year in women.[1] Considering the magnitude of problem, it becomes of paramount importance to take various patient and tumor characteristics into consideration while planning out the treatment plan for the patient. Since chemotherapy has considerable side effects and a single regimen cannot be applied to all the patients because of differences in biological nature in tumor cells it becomes very important to individualize the treatment plan for patients of breast cancer. Anthracycline based chemotherapy is most commonly used first line neoadjuvant chemotherapy in locally advanced breast cancer (LABC). Response to neoadjuvant chemotherapy is evaluated by clinical assessment or ultrasonological imaging, this response is variable in these patients. At present there is no clinically useful marker to predict response of chemotherapy which can be used to identify nonresponders and thus sparing patients from side effects of cytotoxic drugs. Many new biomarkers are being investigated for this purpose. Tumor necrosis factor-α (TNF-α) is one such cytokine which has been found to predict response of neoadjuvant chemotherapy in locally advanced breast cancer.[2] Plasminogen activator inhibitor type 1 (PAI-1) is an inhibitor of Urokinase-type plasminogen activator, its role as a prognostic marker in breast cancer has been studied extensively but its role as predictor of response to neoadjuvant chemotherapy is not clear and requires further studies. The primary aim of this study is to find out the relationship of these markers (PAI-1 and TNF alpha) with response of neoadjuvant chemotherapy so that non-responders can be saved from side effects of chemotherapy and treatment decisions can

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be individualized in locally advanced breast carcinoma patients.

MATERIALS AND METHODS

A prospective study was conducted in the department of surgery in collaboration with department of biochemistry, department of pathology and department of radiodiagnosis & imaging, Maulana Azad Medical College & associated Lok Nayak Hospital and GB pant Hospitals, New Delhi from May 2010 to April 2012. Twenty-five cases of locally advanced breast cancer presenting to surgery department, Lok Nayak Hospital, Delhi fulfilling the inclusion criteria were included after written informed consent and as per STROBE guidelines. However, patients of in situ disease, early breast carcinoma and metastatic breast cancer were excluded from this study along with patients suffering from systemic inflammatory condition.

TNM staging: Clinical staging using TNM classification was performed in all enrolled patients and was based on all information available before first treatment. This included Documenting Tumor size (on USG), node status and metastatic workup including USG abdomen and Chest X Ray. The American Joint Committee on Cancer (AJCC) TNM staging system was used for staging of the patients. Trucut biopsy was done prior to start of chemotherapy for histopathological examination and hormone receptor status of tumor.

TNF-α and PAI-1 levels were measured before commencement of treatment (baseline), after 2 weeks of each cycle of chemotherapy (CAF regimen), and after 2 weeks of surgery. Blood samples were collected pre-chemotherapy, after each cycle of chemotherapy for three cycles and after surgery. TNF-α and PAI-1 was quantified by ELISA.

Chemotherapy administered:

Standard anthracycline based chemotherapy (Cyclophophamide 500 mg/m2, Doxorubicin 50 mg/m2, 5-Fluorouracil 500 mg/m2) was given to all patient as 21 days cycle for three cycles as neoadjuvant chemotherapy.

Histopathologic characterization:

After enrolment into the study, each patient underwent Modified radical mastectomy after receiving neoadjuvant chemotherapy (three cycles). Tissue specimens were examined for the following characteristics: (1.) Presence or absence of invasive carcinoma; (2.) Tumor size: Gross examination of specimen was done after serial sectioning; (3.) Histologic grade: Modified Scarff-Bloom-Richardson grading was used and grade1 to 3 were assigned according to differentiation of tumor; (4.) Estrogen and progesterone receptor and HER-2-Neu status. Axillary lymph nodal tissue was microscopically examined for the presence of malignant cells, number of lymph nodes and lymph nodes positive for metastasis.

Assessment of response to chemotherapy:

Ultrasound examination of the breast mass was performed using high frequency 5-12 MHz transducer. Response to chemotherapy was assessed by measuring the size of mass on serial ultrasound, before and after each cycle of chemotherapy. Clinical response was categorized by using the classification system of the World Health Organization (WHO). The two largest perpendicular diameters of the primary tumor were measured and their product was calculated. Patients were categorized as being in complete remission if there was no clinical evidence of tumor remaining in the breast. A partial response was defined as a reduction in the diameter product of more than 50%. If there was an increase of 25% in the diameter product, the patient was considered to have progressive disease. Patient whose tumor response did not meet the definitions of complete remission, partial response or progressive disease, were considered to have stable disease.

The data was analyzed by using SPSS statistical software version 16.0. P value ≤ 0.05 (2 tailed) was considered to be statistically significant.

RESULTS

Age of the studied patients varies from 28-63years. Most of the patients (60%) were in age group 41-60 years. Mean age in years was 47.32. The 40% (10) of patients were of premenopausal group and 60% (15) of patients were from postmenopausal age group.

[Table 1] summarises changes in changes in Serum TNF alpha and PAI-1 Levels according to advancing stage of disease. The increase in baseline values of TNF alpha with advancing age of disease was found to be significant (p value 0.019) using kruskal wallis test.

Table 1: TNM stage and baseline serum levels of TNF α and PAI-1

TNM Stage	Number (%)	TNFα (Mean) Baseline(pg/ml)	PAI-1 (Mean) Baseline(ng/ml)
T3N1M0	15(60)	26.196 ± 11.986	53.626 ± 2.848
T4bN0M0	3(12)	43.890 ± 14.076	56.333 ± 4.047
T4bN1M0	4(16)	35.762 ± 11.986	55.875 ± 3.277
T4bN2M0	3(12)	47.673 ± 13.728	58.446 ± 2.402
P val*		0.019	0.07

*Kruskal Wallis test

[Table 2] summarises changes in TNFα and PAI-1 level according to increasing Lymph node involvement. The increase in mean value of TNFα

and PAI-1 with increasing Lymph node involvement was found to be non significant (p value 0.068 and 0.086 respectively).

Table 2: Lymph node status and baseline levels of TNFα and PAI-1

Axillary lymph nodes	Number (%age)	TNFα(Mean) Baseline(pg/ml)	PAI-1(Mean) Baseline(ng/ml)
N0	1(4)	8.67	50
N1(1-3)	2(8)	16.60 ± 1.0465	50.380 ± 0.424
N2(4-9)	12(48)	33.840 ± 14.112	55.146 ± 3.308
N3 (10 or more)	10(40)	36.272 ± 12.930	55.971 ± 2.677
P value*	25(100)	0.068	0.086

*Kruskal wallis test

The reduction of TNFα and PAI-1 Level after 3cycles of chemotherapy and surgery were found to be significant (p value 0.000 and 0.01 respectively) using wilcoxan signed rank test as summarized in [Table 3].

Table 3: Baseline, post chemotherapy and after surgery levels of TNFα and PAI-1

	Baseline	Post chemotherapy (After 3 cycles)	After 2 weeks of surgery	P value*
TNFα(pg/ml)	32.427±14.338	19.13±10.762	23.518±12.529	0.000
PAI-1(ng/ml)	54.889±3.305	52.865±3.180	53.411±3.209	0.01

* wilcoxan signed rank test

[Table 4] summarizes changes in TNFα and PAI-1 levels according to ER status. The differences between mean values of PAI-1 according to ER Status was found to be significant (p value 0.023) using Mann Whitney test.

Table 4: ER status and baseline levels of TNFα and PAI-1

Parameter	Number (%age)	TNFα(Mean) Baseline (pg/ml)	PAI-1(Mean) Baseline (ng/ml)
ER +ve	7(28)	40.764 ± 15.452	57.215 ± 2.686
ER -ve	18(72)	29.185 ± 12.892	53.989 ± 3.127
P val.*		0.102	0.023

*Mann Whitney test

[Table 5] summarizes changes in TNFα and PAI-1 levels according to PR status. The differences between mean values of PAI-1 according to PR Status was found to be significant (p value 0.039) using Mann Whitney test.

Table 5: PR status and baseline levels of TNFα and PAI-1

Parameter	Number (%age)	TNFα (Mean) Baseline (pg/ml)	PAI-1(Mean) Baseline (ng/ml)
PR +ve	9(36)	39.577 ± 14.069	56.690 ± 2.637
PR-ve	16(64)	28.405 ± 13.244	53.876 ± 3.275
P val*		0.089	0.039

* Mann Whitney test

[Table 6] Changes in TNFα and PAI-1 levels according to HER2/neu status. The differences between mean values of TNFα and PAI-1 according to HER2/neu status was found to be non significant using Mann Whitney test.

Table 6: HER2/neu status and baseline levels of TNFα and PAI-1

Parameter	Number (%age)	TNFα(Mean) Baseline (pg/ml)	PAI-1(Mean) Baseline (ng/ml)
HER2/neu +ve	17(68)	35.202 ± 13.571	55.367 ± 3.230
HER2/neu - ve	8(32)	26.528 ± 15.018	53.873 ± 3.444
P val*		0.145	0.322

* Mann Whitney test

[Table 7] Changes in TNFα and PAI-1 levels according to Histological grade. The differences between mean values of TNFα and PAI-1 according to Histological grade was found to be significant using Kruskal wallis test.

Table 7: Histological grade and baseline levels TNFα and PAI-1

Histological grade	Number (%age)	TNFα(Mean) Baseline (pg/ml)	PAI-1(Mean) Baseline (ng/ml)
I	8(32)	19.213 ± 6.070	52.688 ± 2.590
II	10(40)	31.208 ± 10.694	54.174 ± 2.738
III	7(28)	49.270 ± 6.447	58.425 ± 1.650
P val*		0.000	0.002

* Kruskal wallis test

[Table 8] summarises the inverse relationship between baseline level TNFα and response to neoadjuvant chemotherapy was statistically significant (P value= 0.001) Pearson Chi-Square test.

Table 8: Baseline levels of TNFα and response to chemotherapy

TNFα (Mean) Baseline (pg/ml)	Number of patients	Complete response	Partial response	Stable disease
<15	2	1	1	0
16-40	13	0	13	0
>40	10			10

Table 9: Baseline levels of PAI-1 and response to chemotherapy

PAI-1 (Mean) Baseline (ng/ml)	Number of patients	Complete response	Partial response	Stable disease
<50	2	1	1	0
50-55	11	0	10	1
55-60	12	0	3	9

[Table 9] summarises the inverse relationship between baseline level PAI-1 and response to neoadjuvant chemotherapy was statistically significant (P value= 0.001) Pearson Chi-Square test. TNFα and PAI-1 was found to be an independent predictive factor for response to chemotherapy, thus providing as a safe, easy, objective and convenient supplement to other known factors in assessing response to chemotherapy.

DISCUSSION

TNF-α has been shown to be involved in cytotoxic destruction of tumor cells in vitro, and due to its elevated levels in malignant diseases, it was put forward that this cytokine might be a useful immunological biomarker in this group of patients.[3-5]

Present study was conducted to find out the relationship of markers (PAI-1 and TNF alpha) with response of neoadjuvant chemotherapy so that non-responders can be saved from side effects of chemotherapy and treatment decisions can be individualized in locally advanced breast carcinoma patients. All these patients had histologically proven locally advanced breast cancer disease. Our intent was to investigate the significance of serum levels of PAI-1 and TNFα as a predictor of response to chemotherapy as well as relationship with other variables such as histological characteristics, lymph node status and immunohistochemistry markers (ER, PR and Her-2neu). Our study showed that there is progressive increase in of PAI-1 and TNFα levels as the stage of disease progresses. This increase is found to be statistically significant for TNFα and non significant for PAI-1. There has not been any previous study which compared serum levels of PAI-1 and TNFα with stage of disease. We found significant decrease in serum levels of PAI-1 and TNFα after the patients received 3 cycles of chemotherapy and this decrease in PAI-1 and TNFα was found to be statistically significant. Tesarova et al[6] have shown that levels of TNF-α decreases as compared to the serum levels before the start of therapy. Taxanes, which induce biological effects especially on the immune system in addition to cytotoxicity, lead to reduction of serum TNF-α levels in those advanced breast cancer patients who respond to chemotherapy.[7] Jablonska et al,[8,9] demonstrated that the serum concentration of soluble TNF receptors drops following adjuvant chemotherapy in patients with breast cancer.

In our study there was statistically significant correlation between serum baseline levels of PAI-1 and ER/PR status, but the same was not true for TNF-α, however with respect to response to neoadjuvant chemotherapy, complete/partial response was seen in 52% of patients with ER –ve status and only 8% in patients with ER +ve status. The results in our study were in line with the retrospective analysis of 1731 patients treated with various neoadjuvant regimens, pCR rates were 24% in patients with ER-negative tumors and 8% in patients with ER-positive tumors regardless of the treatment regimens. Multiple large prospective neoadjuvant clinical trials also showed that pCR rates were significantly higher in patients with hormone receptor negative tumors.[10,11] Ring et al.[12] analyzed their 435 patients who received NACT for operable breast cancer. Patients whose tumors were ER-negative were more likely to achieve a pCR than patients who were ER-positive (21.6% vs. 8.1%). However, ER status and grade did not appear to be independent predictors of pCR. The European Cooperative Trial in Operable breast cancer (ECTO) included a total of 1355 women in the study.[13] Of note, 42% of patients with ER-negative tumors had pathologic complete response vs. 12% in the ER-positive group. In multivariate analysis, ER status emerged as the only independent variable significantly associated with likelihood of achieving a clinically complete response, and most importantly a pCR.

We observed a significant reduction in TNF-α levels in tumors following induction chemotherapy, and the decrease was substantial in complete pathological responders. When serum concentrations of the cytokine in patients with partial and complete responses were compared the difference was statistically significant. The same trend was evident when the primary tumor and axillary lymph nodes were analyzed separately. TNF-α level were significantly reduced in patients achieving partial and complete pathological responses in the regional lymph nodes and primary tumor compared to pre-treatment concentrations. It is important to note that the type of response correlated with the relative changes in serum TNF values. This relation may serve to determine the timing of locoregional treatment after downstaging of patients with locally advanced breast cancer. With TNFα as variable and response to neoadjuvant therapy as state, 30.7 pg/ml at baseline, was derived as cut off value TNFα for to predict response to chemotherapy with sensitivity and specificity of 100% and 93% respectively. Previous studies showed that the mean pre-treatment TNF-α value of breast cancer patients was 15.9±0.9 pg/mL while it was 5.8±1.7 pg/mL in the control group; the difference was statistically significant. The serum levels of TNF-α were markedly decreased in patients with partial and complete responses compared to pre-treatment values. There

was also a difference in TNF-α levels in patients with partial vs complete responses. The relative change between pre- and post-treatment values correlated significantly with the type of response. These results suggest that the serum concentration of TNF-α can be an indicator of response and could be used in clinical decision-making for patients with locally advanced breast cancer.[2]

With PAI-1 as variable and response to neoadjuvant therapy as state, 55.6 ng/ml at baseline, was derived as cut off value PAI-1 for to predict response to chemotherapy under non parametric assumption with sensitivity and specificity of 90% and 86.7% respectively. Previous study showed that PAI-1 levels alone were able to discriminate responders from nonresponders with 75% sensitivity and 77% specificity prior to the start of NAC. Several groups have illustrated experimentally the role of tPAI-1 in tumor progression and its negative prognostic value in breast cancer.[11-15]

CONCLUSION

TNFα and PAI-1 are easily available biomarker which can be combined with the conventional predictive markers in locally advanced breast cancer. Significant post chemotherapy decrease in serum TNFα and PAI-1 levels may provide objective criteria to assess response to chemotherapy. Serum TNFα and PAI-1 levels may be used as yardstick for systemic neoadjuvant therapy in locally advanced breast cancer. TNFα and PAI-1 may prove to be a safe, convenient and easily available biomarker which can be combined with the conventional predictive markers in locally advanced breast cancer. These findings need to be validated with large multicentered prospective studies with long term follow up.

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