International Journal of Surgery 10 (2012) 638–640

Contents lists available at SciVerse ScienceDirect

International Journal of Surgery

journal homepage: www.theijs.com

Original research

Effect of standard anthracycline based neoadjuvant chemotherapy on circulating levels of serum IL-6 in patients of locally advanced carcinoma breast – A prospective study

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ARTICLE INFO

Article history:
Received 2 July 2012
Received in revised form
19 October 2012
Accepted 6 November 2012
Available online 16 November 2012

Keywords:
Interleukin 6 (IL-6)
Breast carcinoma
Chemotherapy

ABSTRACT

Background: Elevated IL-6 levels have been associated with advanced stage of breast cancer and metastasis-related morbidity. The present prospective study was carried out to assess the effect of neoadjuvant chemotherapy on circulating levels of serum IL-6 in patients of locally advanced carcinoma breast.

Materials: All locally advanced carcinoma breast cases presenting to the surgery out patient's department were included in the study excluding pregnant or lactating females and those patients who were unfit for anthracycline based chemotherapy. A total of 30 cases were included. The various parameters that were evaluated include detailed clinico-pathological profile and IL-6 levels. Clinical staging using TNM classification was performed in all enrolled patients. This included documenting tumor size (on USG), node status and metastatic workup. First blood sample was collected before start of any treatment. Second blood sample was collected after 3 cycles of chemotherapy. Blood was centrifuged within 30 min and serum kept at −80 °C until analysis for IL-6. IL-6 levels were quantified by ELISA.

Results: Majority of patients presented in stage T3N1M0 (66.66%). The serum level of IL-6 increased as the disease progressed from T3N1M0 to T4dN2M0 (41.4 ± 31.9 pg/ml vs. 164.0 ± 31.1 pg/ml respectively). A progressive reduction in IL-6 levels with subsequent cycles of chemotherapy was observed which was statistically significant (from 72.8 ± 56.0 pg/ml to 47.0 ± 61.9 pg/ml; *p* value 0.002 wilcoxan signed rank test).

Conclusion: Our study shows a consistent decline in the IL-6 levels with chemotherapy. Upon ratification of our findings by large population based multi centric studies, we may state with conviction that a single blood test as serum level of IL6 will prove beneficial in assessing the efficacy of chemotherapy.

1. Introduction

A wide repertoire of cytokines is secreted by the breast tumors with IL-6 being one of them.[1] The involvement of IL-6 at the cellular level is reflected by the results of serum studies of cancer patients, where IL-6 has been proven to be a valuable indicator of prognosis and tumor load. Elevated IL-6 levels have been associated with advanced stage of the disease and metastasis-related morbidity. There are studies showing rise in IL-6 levels with increasing tumor size, lymph node involvement, adipose tissue invasion and distant metastasis.[2–4] It can now be fathomed that high IL-6 serum levels provide an environment that is favorable to tumor growth.[5]

Neoadjuvant chemotherapy is often used preoperatively to decrease the tumor load in patients of locally advanced carcinoma breast. Since serum IL-6 levels are directly correlated to tumor load, it is expected that there should be a fall in these levels with decreasing tumor load. The present prospective study was carried out to assess the effect of neoadjuvant chemotherapy on circulating levels of serum IL-6 in patients of locally advanced carcinoma breast.

2. Materials and methods

A prospective study was conducted in the Department of Surgery in collaboration with Department of Biochemistry, Maulana Azad Medical College and associated Lok Nayak Hospital and G B Pant Hospitals, New Delhi from May 2010 to April 2011. All locally advanced carcinoma breast cases presenting to the surgery out patient's department were included in the study excluding pregnant or lactating females and those patients who were unfit for anthracycline based chemotherapy. A

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http://dx.doi.org/10.1016/j.ijsu.2012.11.007

total of 30 cases were included. The study was commenced following detailed patient consent and clearance by the institutional ethical committee.

The various parameters that were evaluated include detailed clinico-pathological profile and IL-6 levels. Clinical staging using TNM classification was performed in all enrolled patients. This included documenting tumor size (on USG), node status and metastatic workup. The American Joint Committee on Cancer (AJCC) TNM staging system was used for staging of the patients.

First blood sample was collected before start of any treatment. 5 ml of blood was drawn from the antecubital vein of the patient after applying tourniquet to arm with 24 gauge needle in the morning. Venous blood was collected in a plain vial. After enrollment into the study, each patient received 3 cycles of neoadjuvant chemotherapy (Cyclophosphamide, adriamycin and 5-fluorouracil based) at 3 weekly interval. Second blood sample was collected after 3 cycles of chemotherapy. Blood was centrifuged within 30 min and serum kept at −80 °C until analysis for IL-6. IL-6 levels were quantified by ELISA.

2.1. 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.

All patients underwent modified radical mastectomy after neoadjuvant chemotherapy. Tissue specimens were examined for evidence of invasion, tumor size, mitotic index, histologic grade, receptor status, axillary lymph node status and adipose tissue invasion.

2.2. Statistical analysis

The data was analyzed by using SPPS statistical software version 16.0. Wilcoxan signed rank test was used to determine the statistical significance on different points of time within the same group.

3. Results

Age of patients varied from 32 to 66 years. The mean age in years was 47.7yrs. Stage distribution of patients is depicted in Fig. 1. Majority of patients presented in stage T3N1M0 (66.66%). The 43.3% ($n = 13$) patients were of premenopausal age group and 56.6% ($n = 17$) patients were of postmenopausal age group.

The serum level of IL-6 increased as the disease progressed from T3N1M0 to T4dN2M0 (41.4 ± 31.9 pg/ml vs. 164.0 ± 31.1 pg/ml respectively) (Fig. 2).

Overall mean value of baseline serum IL-6 in this study was 72.8 ± 56.0 pg/ml. After 3 cycles of chemotherapy this value came down to 47.0 ± 61.9 pg/ml.

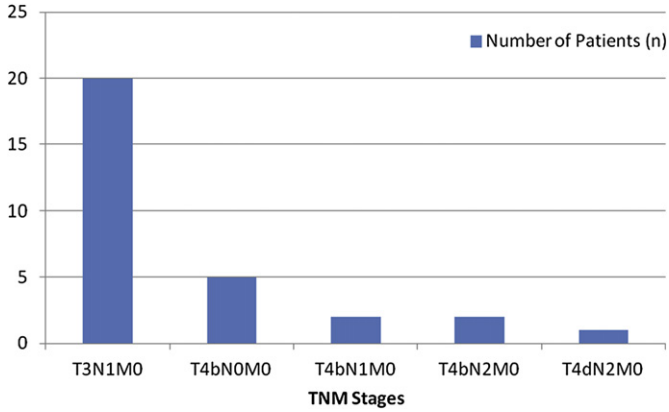

Fig. 1. Stage-wise distribution.

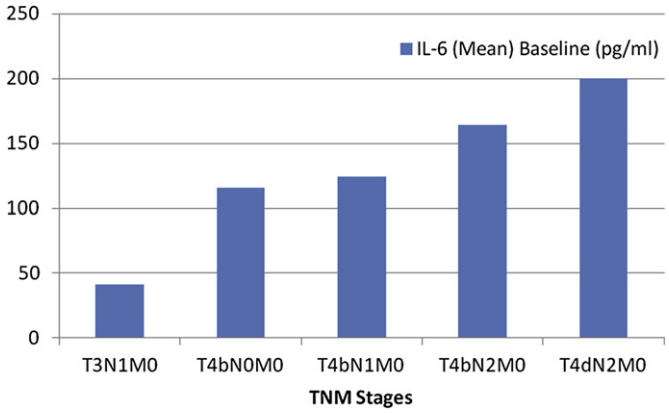

Fig. 2. Distribution of IL-6 levels according to stage of the disease.

The reduction in IL-6 values after 3 cycles of chemotherapy were significant for IL-6 (p value 0.002 wilcoxan signed rank test) as summarized in Table 1.

The fall in IL-6 levels with neoadjuvant chemotherapy were significant for both T3 and T4 tumors. In T3 tumors, levels dropped from 41.4 ± 31.9 to 29.6 pg/ml whereas in T4 tumors, levels changed from 135.5 ± 37.5 to 72.5 ± 56.03 pg/ml ($p < 0.05$).

Partial or complete response to chemotherapy was seen in 19 patients whereas disease was stable or progressive in 11 patients. Those who responded well to chemotherapy also showed a significant decline in IL-6 levels (from 55.2 ± 44.1 to 14.3 ± 12.9 pg/ml; $p < 0.05$). In patients having stable or progressive disease, there was no significant fall in IL-6 levels (from 107.6 ± 59 to 104.5 ± 71.3 pg/ml) (Table 2).

There was a statistically significant correlation of IL-6 levels with advancing axillary lymph node involvement, histologic grade, adipose tissue invasion and mitotic index. The levels were 102.0 ± 68.3 and 55.8 ± 40.5 pg/ml in tumors with mitotic index 2 and 1 respectively.

4. Discussion

Efficacy of neoadjuvant chemotherapy in the management of breast cancer in particular, is beyond doubt in locally advanced breast cancer; primarily due to its ability to downsize large tumors.[6] Several randomized prospective studies on breast cancer patients have proved the safety and efficacy of neoadjuvant chemotherapy.

Cytokines regulate function of many cells in an additive, synergistic or antagonistic manner. Interleukin-6 (IL-6) is mainly secreted by fibroblasts, macrophages and lymphocytes (mainly TH2 cells). It is a multi-poietic cytokine that promotes the growth and differentiation of immune cells, the production of other cytokines, and acute-phase protein synthesis. It promotes tumor growth by up-regulating antiapoptotic and angiogenic proteins in tumor cells.[7–9] IL-6 is associated with angiogenesis by virtue of its ability to induce the mRNA of vascular endothelial growth factor (VEGF),

Table 1
Baseline and post chemotherapy levels of IL-6.

	Baseline	Post chemotherapy (3 cycles)	p value[a]
IL-6 (pg/ml)	72.7 ± 56.0	47.0 ± 61.9	**0.002**

Bold value represents $p < 0.05$.
[a] Wilcoxan signed rank test.

Table 2
Changes in IL-6 levels according to clinical response.

	Partial/complete response	Stable/progressive disease
Number of patients	19	11
IL-6 (Pre chemotherapy) (pg/ml)	55.2 ± 44.1	107.6 ± 59
IL-6 (Post chemotherapy) (pg/ml)	14.3 ± 12.9	104.5 ± 71.3

which is typically a direct angiogen.[10] Serum interleukin-6 values have been shown to be significantly more in patients with breast cancer as compared to normal healthy women, which was correlated with clinical stage of disease and poor survival.[2–4] Production of IL6 is up-regulated by cytokines such as TNF and IL-1, as well as by certain oncogenes.[11]

In our previous study, we found a direct correlation of serum IL-6 levels with stage, lymph node metastasis and adipose tissue invasion.[2] This study was carried out to find out the effect of anthracycline based chemotherapy on these levels; fall in which will further strengthen the correlation of IL-6 levels with tumor load. No such study has been carried out on Indian patients to the best of our knowledge.

Very few studies are available in literature that has evaluated the effect of CAF regimen on IL-6 levels. We found significant decrease in serum levels of IL-6 after the patients received 3 cycles of chemotherapy concordant with a similar study done by Yokoe et.al.[12] On the contrary, Chala et al., found no effect of chemotherapy on these levels in a small subset of 10 patients (stage 4).[13] Another study by Tsavaris et al., evaluated the effect of taxane therapy on cytokine levels. They reported a rise in IL-6 levels with progression of chemotherapy. This may be attributed to the immunomodulatory effect of taxanes on the T cells.[14]

There are a few limitations of our study. Firstly, the small sample size is a major impediment. A larger sample size with appropriate representation of each stage would have been preferable for better interpretation of our data. Secondly, a long term follow up would have enabled us to correlate the IL6 levels with recurrence and 5 year survival rates. These could not be achieved due to managerial and financial constraints.

5. Conclusion

Cytokines have been proven to be mediators in the entire spectrum of carcinogenesis since the initiation to final metastatic spread. Our study shows a consistent decline in the IL-6 levels with chemotherapy. Upon ratification of our findings by large population based multi centric studies, we may state with conviction that a single blood test as serum level of IL6 will prove beneficial in assessing the efficacy of chemotherapy.

Ethical approval

Ethical approval was taken from institutional ethical committee.

Funding

None declared.

Author contribution

Dr Nikhil Gupta: study design, data analysis, writing manuscript.

Dr Binita Goswami: study design, data collection, writing manuscript.

Dr Pankaj Mittal: data collection, data analysis.

Conflict of interest

None of the authors has any financial ties or conflicts of interest to disclose.

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