Journal of Cancer Research and Therapeutics

October-December 2007 | Volume 3 | Issue 4

CONTENTS

Original Article

Free full text available from
www.cancerjournal.net

Estimation of BCL-2 protein in carcinoma of the breast and its clinical correlation in locally advanced breast cancer

Himanshu Aggarwal, Parvinder S Lubana, DK Jain, RK Mathur

Department of Surgery, M.G.M. Medical College and M.Y. Hospital Indore - 452 001, India

For correspondence: Dr. Himanshu Aggarwal, Department of Surgery, M.G.M. Medical College and M.Y. Hospital, Indore - 452 001, India. E-mail: himagg123@yahoo.com

ABSTRACT

The change in expression of apoptotic markers (Bcl-2 and Bax proteins) brought about by various chemotherapeutic regimens is being used for its predictive value for assessing response to neoadjuvant chemotherapy (NACT) in locally advanced breast carcinoma (LABC).

Aims: (1) Estimation of Bcl 2 expression in LABC, (2) Any change in Bcl 2 expression following chemotherapy in LABC, (3) Any relation of Bcl 2 estimation to changes in size of tumor, nodal status, age, and menopausal status.

Settings and Design: This was a prospective study of 120 cases of LABC.

Materials and Methods: All cases were subjected to biopsy and the tissue was evaluated immunohistochemically for apoptotic marker Bcl-2 family protein. Three cycles of NACT were given at three-weekly intervals. Modified radical mastectomy was performed and the specimens were re-evaluated for any change in the Bcl-2 family protein. The clinical response and immunohistochemical response were correlated and compared.

Statistical Analysis: Coefficient of correlation was calculated by Pearson correlation coefficient (*P*-value).

Results: Clinical response, as measured by reduction in the tumor size, was observed in 81 (67.5%) patients while immunohistochemical response was observed in 67 (55.8%) patients. Correlation between immunohistochemical and clinical response was found to be statistically significant ($P = 0.02$). Nodal response was seen in 72 (60%) patients. There were no patients in the N_0 group; 22 (53.7%) of the N_1 patients were down-staged to N_0, while 19 (46.3%) remained N_1. In patients with N_2 disease, 11 (13.9%) were down-staged to N_0 status, 39 (49.4%) were down-staged to N_1 status, and 29 (36.7%) did not show any response. Immunohistochemical response was observed in 67 (55.8%) patients. Correlation between immunohistochemical and nodal responses was also found to be statistically significant ($P = 0.03$).

Conclusions: This significant positive correlation between clinical and immunohistochemical responses highlights the importance of apoptotic marker Bcl-2 family protein in predicting the response of LABC to NACT.

KEY WORDS: Apoptosis, Bcl-2, neoadjuvant chemotherapy

INTRODUCTION

Advances in molecular biology have enabled researchers to examine the mechanisms of neoplastic proliferation at the level of both the nucleus and the genes. Neoadjuvant chemotherapy (NACT) is an essential part of the multidisciplinary approach to the management of locally advanced breast cancer (LABC). It is required for both local control as well as for systemic control.[1-4] Recognition that tumor growth rate is a product of the proliferative activity and the rate of cell death, has led to a reappraisal of the traditional views regarding tumor response and resistance to cytotoxic drugs.[2] Mechanisms that suppress apoptosis may be important in the development of intrinsic and acquired resistance to cytotoxic drugs.[3] The proto-oncogene Bcl-2 encodes a protein that inhibits apoptosis, a common mechanism of cell death caused by hormone and chemotherapy.

Bcl-2 is an inhibitor of apoptosis and is overexpressed in more than half of all human cancers. Overexpression of Bcl-2 occurs in 40-80% of human breast tumors. In laboratory studies, ectopic overexpression of the anti-apoptotic protein Bcl-2 has been shown to result in resistance to the cytotoxic effects of many chemotherapeutic drugs.[5] Bcl-2 and Bax proteins indicate a combined consideration of proteins that have been found to negatively and positively regulate apoptosis in translational studies on the effect of chemical and physical agents at a cellular level.[6] Various other biological markers, such as P-glycoprotein expression, have also been used to predict the response to NACT.[7] Studies have shown that decrease in Bcl-2 expression after chemotherapy,

relative to the expression from the pretreatment sample, correlates with disease response.[8]

The aim of this study was to find out if any relationship exists between immunohistochemical response (change in expression of apoptotic marker Bcl-2 protein) and clinical response after chemotherapy.

MATERIALS AND METHODS

This was a prospective study that was conducted from January 2000 to April 2006. Patients' ages ranged from 28-75 years, with a mean age of 55.4 years.

After taking permission from the local ethics committee and obtaining detailed informed written consent, 120 histopathologically proven (according to the 6th edition of AJCC (American Joint Committee on Cancer classification) cases of LABC were included in the study. A thorough clinical and ultrasonographic (USG) examination of all the patients, including examination of the opposite breast, was performed to stage the disease accurately. Routine and metastatic workup was done, including complete blood examination (total blood count and platelet count), chest radiograph, ECG (with echocardiography done whenever ECG showed a positive finding), liver function tests, bone scan, USG abdomen, and KFT (kidney function tests). Approximate tumor size and axillary lymph node status were recorded at baseline, before NACT. A core biopsy using a Tru-Cut needle/incisional biopsy was performed for semiquantative immunohistochemical estimation of the apoptotic marker, i.e., baseline Bcl-2, before initiating the chemotherapy. Patients were subjected to three cycles of CAF regime (cyclophosphamide 600 mg/m^2, adriamycin 50 mg/m^2, and 5-fluorouracil 600 mg/m^2) at intervals of three weeks. Before each cycle, the patient was clinically and sonographically examined for breast tumor size, axillary lymph node status, and appearance of systemic metastasis. All patients were subjected to modified radical mastectomy three weeks after the last cycle and the specimens were again subjected to semiquantative immunohistochemistry to evaluate for any change in the Bcl-2. Clinical response was defined as >50% reduction in the tumor size after completion of three cycles of NACT, as assessed clinically and by sonographic evaluation. Those showing less than 50% reduction in tumor size, both clinically and by sonographic evaluation, were considered as nonresponders.

Any decrease in nodal staging (i.e., N_1 to N_o or N_2 to N_1-N_o) was taken as indication of a responder. Immunohistochemical response was taken as decrease in the Bcl-2 ratio, i.e., post/pre-chemotherapy <1. Any increase or absence of change in this ratio was considered as indication of a nonresponder.

Immunohistochemical methods

The biopsy specimens were kept in buffered formalin solution. 5 Micron sized sections were prepared from paraffin-embedded blocks on glass slides. Sections were deparaffinized in xylene for 15 min and hydrated in alcohol for 20 min. Further, incubation was done in 0.3% hydrogen peroxide in methanol solution for 45 min. The slides were washed with citrate buffer and kept in a water bath at 90-95°C for 45 min for antigen retrieval. Sections were washed with Tris-buffered saline solution and incubated with blocking antibodies (DAKO monoclonal mouse antihuman Bcl-2 oncoprotein for Bcl-2 expression) at 37°C. Incubation with avidin-biotin complex was done at 37°C for 1 h before washing with Tris-buffered saline solution. 3,3 Diaminobenzidine tetrahydrochloride solution was applied for 3-5 min. The slides were then counterstained with hematoxylin solution, washed with distilled water, air dried, and mounted. Slides were examined by conventional immunofluorescence microscopy and were labeled as per the given scoring system. For Bcl-2, positive controls were the mantle zone of lymphoid follicles and the negative controls were the test slides without primary antibody. These were interpreted on the basis of the following criteria:

1. Percentage of cells showing immune bodies:
   - <5% : score 0
   - 5-25% : score 1
   - 25-75% : score 2
   - >75% : score 3
2. Intensity of staining
   - Mild : score 1
   - Moderate : score 2
   - Intens : score 3

There was a strong correlation between the intensity of staining and the percentage of cells showing immune bodies; the percentage of cells showing immune antibodies was considered for the semiquantitative assessment of Bcl-2 family protein.

Statistics

Significance of correlation between various variables was assessed by Pearson correlation coefficient (*P*-value).

RESULTS

One hundred and twenty cases of LABC were included in this study. The mean age of the patients was 46 years (range: 29-65 years); 68 patients were premenopausal.

Sizes of the tumors were measured clinically as well as by ultrasound and the patients were subdivided into four groups [Table 1]. According to the axillary lymph node status the patients were divided into three groups [Table 2].

Clinical response, as measured by reduction in the tumor size, was observed in 81 (67.5%) patients, while immunohistochemical response was observed in 67 (55.8%) patients. The correlation between immunohistochemical and clinical responses was found to be statistically significant ($P = 0.02$) [Table 3].

Table 1: Clinical (tumor size) status (*n* = 120)

Staging	Number of patients (%)
<5 cm	0 (0)
5-8 cm	68 (56.7)
8-10 cm	32 (26.7)
>10 cm	20 (16.7)

Table 2: Lymph node status (*n* = 120)

Staging	Number of patients (%)
N_0	0 (0)
N_1	41 (34.2)
N_2	79 (65.8)

Table 3: Clinical (tumor size) *vs* immunohistochemical response (*n* = 120)

	Clinical (tumor size) response		
	Yes	No	
Immunohistochemical response			
Yes	51 (42.5)	16 (13.3)	67 (55.8)
No	30 (25)	23 (19.2)	53 (44.2)
Total	81 (67.5)	39 (32.5)	120

Figures in parentheses are in percentage, (*P* value = 0.02)

Table 4: Nodal *vs* immunohistochemical response (*n* = 120)

	Nodal response		
	Yes	No	
Immunohistochemical response			
Yes	46 (38.3)	21 (17.5)	67 (55.8)
No	26 (21.7)	27 (22.5)	53 (44.2)
Total	72 (60)	48 (40)	120

Figures in parentheses are in percentage, (*P* value = 0.03)

Nodal response was seen in 72 (60%) patients. There were no patients in the N_0 group and 22 (53.7%) of the N_1 patients were down-staged to N_0, while 19 (46.3%) remained as N_1. In patients with N_2 disease, 11 (13.9%) were down-staged to N_0 status, while 39 (49.4%) were down-staged to N_1 status and 29 (36.7%) did not show any response.

Immunohistochemical response was observed in 67 (55.8%) patients. Correlation between immunohistochemical and nodal responses was also found to be statistically significant ($P = 0.03$) [Table 4].

No significant correlation was found between age and menopausal status with clinical or immunohistochemical response.

DISCUSSION

LABC is the most common (i.e., 30-40%) presentation of breast carcinoma in developing countries, especially in Southeast Asia. In India, the incidence of LABC at first presentation to surgical clinics is estimated to be as high as 25-30%.[2-4]

The realization that breast cancer is not a localized disorder but has widespread systemic manifestations has lead to the opening up of new avenues of treatment. It was discovered very early in the history of breast cancer that patients with locally advanced cancer are likely to harbor undetectable local and systemic micrometastasis at presentation. This has led to systemic treatment assuming greater importance in the multidisciplinary approach. Various studies have been undertaken to prove the efficacy of NACT in terms of clinicopathological and overall response. Jacquillat *et al.*, in a study on 250 patients, with disease stage ranging from stage I to stage IIIB, and using a vinblastine (V), thiotepa (T), methotrexate (M) and 5-fluorouracil (F) (VTMF) regime, with or without adriamycin (A), reported chemotherapy-induced partial clinical response in 41% and overall response in 71%.[9] NACT has also shown benefits in operable breast cancers by increasing the chances of breast conservation by up to 90% in some trials.[7,10,11] The other important advantage of NACT is that it represents an *in vivo* chemosensitivity test for assessment of tumor response from which prognostic information can be obtained. It provides an early treatment for micrometastatic disease, counteracting the increased growth rate, which is possibly determined by the shrinkage of the tumor. The down-staging converts an inoperable case into one amenable to curative resection.[7,10,11]

For every cell, there is a time to live and a time to die. There are two ways in which cells die, i.e., they are either induced to commit suicide or are killed by an injurious agent.

The pattern of events in death by suicide is so orderly that the process is often called programmed cell death or PCD. The cellular machinery of PCD turns out to be as intrinsic to the cell as, say, mitosis. PCD is also called apoptosis. Certain biochemical and genetic events, like DNA fragmentation via end nuclease activation and cleavage of intracellular proteins, expression of Bcl-2 family members, tumor suppressor gene p53-directed events, proto-oncogene activation, and activation of transmembrane receptor signaling pathways such as tumor necrosis factor, control apoptosis.

A wide range of anticancer drugs with widely differing modes of action have been demonstrated to induce apoptosis *in vitro*, suggesting that this is a significant common final pathway through which they exert their clinical effect. Furthermore, the mechanisms that suppress apoptosis may be important in the development of acquired resistance to cytotoxic drugs. Apoptosis or PCD plays an important role in the regulation of tissue development, differentiation, and homeostasis. It is therefore possible that deregulation of apoptosis contributes to the pathogenesis of cancer.[3,11-13] The heterogeneous nature of breast cancer has resulted in overwhelming interest in the search for prognostic markers like Bcl-2, bax, bcl-xL, bag-1, fas, fasL, and p53 that may help identify the patients who might benefit most from the therapeutic modalities available.

Bcl-2 is an inhibitor of apoptosis and is overexpressed in more than half of all human cancers. Overexpression of Bcl-2 occurs in 40-80% of human breast tumors. In laboratory studies, ectopic overexpression of the antiapoptotic protein Bcl-2 has been shown to result in resistance to the cytotoxic effects of many chemotherapeutic drugs.[5]

Bcl-2 and Bax proteins indicate a combined consideration of proteins that have been found to negatively and positively regulate apoptosis in translational studies on the effect of chemical and physical agents at a cellular level.[6]

In a study conducted by Mirjolet *et al.*, in which Bcl-2 levels were analyzed in the cell line after exposure to 5-FU equitoxic concentration, it was found that the Bcl-2 relative expression ratio correlates with 5-FU sensitivity.[14] They concluded that 5-FU sensitivity was related to Bcl-2, and Bcl-2 could be a relevant marker to predict 5-FU treatment response.

In our study, 67.5% of the patients had an objective clinical response following NACT and 55.8% patients had immunohistochemical response. Twenty-five percent of the patients showed clinical response but were immunohistochemical nonresponders. Nodal response was seen in 60% patients and 21.7% showed nodal response but were immunohistochemical nonresponders. Immunohistochemical response, clinical response, and the correlation between the two following NACT, was found to be statistically significant. Clinical response to chemotherapy was found to be independent of the initial tumor size.

During the course of the study, down-staging of nodal status after NACT was found to be significant ($P = 0.03$), which is in contrast to a study conducted by Kuerer et al. (conducted at MD Anderson Cancer Institute in 372 cases of LABC), in which association between pre- and post-NACT response and axillary nodal response was not significant.[15]

CONCLUSION

Induction NACT is an integral part of the multidisciplinary approach in the treatment of LABC. This study highlights the importance of apoptotic marker Bcl-2 family protein in predicting the response to NACT in carcinoma breast. In view of the significant correlation with the clinical response, it can been accepted as a surrogate marker for assessing the efficacy of the chemotherapeutic regimes.

REFERENCES

1. Paymaster JC. Cancer of the breast in Indian women. Surgery 1956;40:372-7.
2. Ellis PA, Smith IE, Detre S, Burton SA, Salter J, A'Hern R, *et al*. Reduced apoptosis and proliferation and increased Bcl-2 in residual breast cancer following preoperative chemotherapy. Breast Cancer Res Treat 1998;48:107-16.
3. Kerr JF, Winterford CM, Harmon BV. Apoptosis: Its significance in cancer and cancer therapy. Cancer 1994;73:2013-26.
4. Jussawalla DJ, Yeole BB, Natekar MV, Narayan RA. Epidemiology of breast cancer in India. Indian J Cancer 1975;12:231-42.
5. Poelman SM, Adeyanju MO, Robertson MA, Recant WM, Karrison T, Fleming GF, *et al*. Human breast cancer susceptibility to paclitaxel therapy is independent of Bcl-2 expression. Clin Cancer Res 2000;6:4043-8.
6. Daidone MG, Luisi A, Veneroni S, Benini E, Silvestrini R. Clinical studies of Bcl-2 and treatment benefit in breast cancer patients. Endocr Relat Cancer 1999;6:61-8.
7. Eltahir A, Heys SD, Hutcheon AW, Sarkar TK, Smith I, Walker LG, *et al*. Treatment of large and locally advanced breast cancers using neoadjuvant chemotherapy. Am J Surg 1998;175:127-32.
8. Buchholz TA, Davis DW, McConkey DJ, Symmans WF, Valero V, Jhingran A, *et al*. Chemotherapy-induced apoptosis and Bcl-2 levels correlate with breast cancer response to chemotherapy. Cancer J 2003;9:33-41.
9. Jacquillat C, Weil M, Baillet F, Borel C, Auclerc G, de Maublanc MA, *et al*. Results of neoadjuvant chemotherapy and radiation therapy in the breast-conserving treatment of 250 patients with all stages of infiltrative breast cancer. Cancer 1990;66:119-29.
10. Buzdar AU, Singletary SE, Booser DJ, Frye DK, Wasaff B, Hortobagyi GN. Combined modality treatment of stage III and inflammatory breast cancer MD: Anderson Cancer Center experience. Surg Oncol Clin North Am 1995;4:715-34.
11. Singh G, Singh DP, Gupta D, Muralikrishna BV. Neoadjuvant chemotherapy in locally advanced breast cancer. J Surg Oncol 1996;61:38-41.
12. Blomqvist C, Elomaa I, Rissanen P, Hietanen P, Nevasaari K, Helle L. Influence of treatment schedule on toxicity and efficacy of cyclophosphamide, epirubicin and fluorouracil in metastatic breast cancer: A randomized trial comparing weekly and every-4-week administration. J Clin Oncol 1993;11:467-73.
13. Kymionis GD, Dimitrakakis CE, Konstadoulakis MM, Arzimanoglou I, Leandros E, Chalkiadakis G, *et al*. Can expression of apoptosis genes, bcl-2 and bax, predict survival and responsiveness to chemotherapy in node-negative breast cancer patients? J Surg Res 2001;99:161-8.
14. Mirjolet JF, Barberi-Heyob M, Didelot C, Peyrat JP, Abecassis J, Millon R, *et al*. Bcl-2/Bax protein ratio predicts 5-fluorouracil sensitivity independently of p53 status. Br J Cancer 2000;83:1380-6.
15. Kuerer HM, Newman LA, Smith TL, Ames FC, Hunt KK, Dhingra K, *et al*. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. J Clin Oncol 1999;17:460-9.

Source of Support: Nil, **Conflict of Interest:** None declared.