Original Article

Immunohistochemical expression of carcinoembryonic antigen-related cell adhesion molecules 5, CEACAM6, and SLC7A5: Do they aid in predicting the response to neo-adjuvant chemotherapy in locally advanced breast cancer?

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ABSTRACT

Context: Neo-adjuvant chemotherapy (NACT) has become an integral part of multimodality treatment for locally advanced breast cancer (LABC) worldwide. Predictors of therapeutic response to NACT are lacking. Whether carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) like CEACAM5 and CEACAM6 can act as a predictor of response to therapy is unclear. SLC7A5 gene in humans encodes a large neutral amino acid transporter protein, which has an essential role in tumor cell growth and survival. **Materials and Methods:** Thirty histopathologically proven cases of LABC, being given NACT, were included in the study. Immunohistochemical examination of the tumor sections was performed for CEACAM5, CEACAM6, and SLC7A5. Response to chemotherapy was assessed using "Response Evaluation Criteria in Solid Tumors" (RECIST) 1.1 criteria. A total of three cycles were given at 3 weekly intervals. After 3 weeks of the last cycle of NACT, the patients were taken up for modified radical mastectomy. The specimen was subjected to histopathological examination. The immunohistochemical results were correlated with response to NACT based on RECIST criteria and histopathology. **Results:** 12/30 (40%) of the patients had objective clinical response of which 4 (13.33%) patients had pathological complete response. The relationship between CEACAM5 and CEACAM6 and response to NACT was found to be statistically significant, $P = 0.004$ and $P = 0.020$, respectively. Furthermore, relationship between response to NACT and node-positive tumors with SLC7A5 immunoreactivity was found to be highly significant ($P = 0.009$). **Conclusion:** Biomarkers (CEACAM5, CEACAM6, and SLC7A5) showed promise as predictors of poor response to NACT and can help plan an alternative regime in likely nonresponders to prevent the toxicity of chemotherapy and also in tailoring the therapy in a patient with LABC.

Key words: Breast cancer, carcinoembryonic antigen-related cell adhesion molecules 5, carcinoembryonic antigen-related cell adhesion molecules 6, neo-adjuvant chemotherapy, SLC7A5

INTRODUCTION

Breast cancer is the leading cause of cancer mortality in women worldwide. In India and in other developing countries, up to 25–30% of patients present as locally advanced breast cancer (LABC).[1] An increasing trend in the incidence rates of breast cancer has been reported from the various registries of National Cancer Registry Project.[2] The management of LABC has changed over decades from primarily local modalities to regimens that combine systemic and local therapy. It was the realization that patients with LABC are likely to have undetectable micrometastases at diagnosis that led to systemic treatment assuming the major focus of the multimodality approach.[3] The studies have confirmed that surgery alone is an inadequate treatment in the management of patients with LABC. Even aggressive surgical techniques in patients with advanced local disease

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have shown a high-incidence of local recurrence. Most importantly, surgery did not change the pattern of distant failure in these patients who probably had micrometastatic disease at the time of diagnosis.[3] Presently, neo-adjuvant chemotherapy (NACT) with interval debulking surgery and postsurgery chemotherapy, is also preferred for advanced stage disease of serous ovarian cancers (Stage IIIc or IV, of the International Federation of Gynecology and Obstetrics staging system).[4]

One of the recommended protocols for management of LABC presently is NACT with three cycles of CAF regime (CMF regime in cardiotoxic patients) followed by modified radical mastectomy (MRM) and subsequently three or more cycles of adjuvant chemotherapy with or without hormone therapy and/or radiotherapy.[5-7] Down staging of the tumor achieved with NACT facilitates optimum surgery ensuring R0 resection (microscopically tumor-free margins).[5] NACT represents an *in vivo* chemosensitivity test for assessment of tumor response to a particular regime from which prognostic information for further treatment regimens can be obtained. While some patients show partial or complete response to the above drugs in the form of decrease in tumor size, and/or down staging of lymph node status, others fail to do so. Development of resistance to chemotherapeutic agents is a major and evolving problem.[8-11] The above drugs for NACT are potentially toxic to the patient with serious side-effects and need close monitoring.[12] Thus, to be able to predict the response before initiating chemotherapy has always been a challenge. Breast cancer is a heterogeneous disease, and there is a continual drive to identify markers that will aid in predicting response to therapy. While ER/PR status of the tumor has been accepted as a predictive parameter for response to hormone and chemotherapy in breast cancer, the search for more sensitive biomarkers is still on. Thus, studying the biological markers to predict response to NACT may permit tailoring of regimens to achieve maximal tumor response in a particular patient.

The role of carcinoembryonic antigen-related cell adhesion molecules 5 and 6 (CEACAM5 and CEACAM6) is not yet very well-established in predicting response to therapy in carcinoma breast.[13,14] SLC7A5 is the gene for solute carrier family 7 which is a cationic amino acid transporter found on chromosome 16 with expression in various normal tissues as well as tumor tissues. By gene expression pattern, it clusters with markers of proliferation in breast cancer and has been previously noted to be highly expressed in some cancers.[15] CEACAM5, CEACAM6, and SLC7A5 determination may give additional predictive information on the behavior of tumor tissue to NACT as well as antibody treatment in breast cancer. Against this background, a prospective study was contemplated with the hypothesis that these biomarkers can help predict the response to NACT in LABC patients.

MATERIALS AND METHODS

Thirty cases of LABC (Stage IIb and III) were included in the study. A written informed consent was taken from all patients for inclusion in the study. The study was approved by the Institutional Ethics Committee. The histopathological diagnosis was based on a core needle biopsy. The biopsy was also assessed for immunohistochemical expression of CEACAM5, CEACAM6, and SLC7A5. All patients underwent ultrasonography/mammography/magnetic resonance imaging and metastatic workup (ultrasound abdomen, bone scan, and diagnostic chest computed tomography scans) for the accurate assessment of the stage of the disease. Patients were evaluated for fitness for receiving NACT based on blood investigations (complete blood count, kidney function tests) and cardiac evaluation (electrocardiogram and two-dimensional echocardiography). All patients received three cycles of NACT in the form of CAF regime (cyclophosphamide – 500 mg/m^2, adriamycin – 50 mg/m^2, and 5-fluorouracil – 500 mg/m^2) at 3 weekly intervals. Prior to each CAF cycle, patients underwent clinical as well as ultrasonological assessment of tumor size and axillary lymph nodes. Response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) (1.1)[16] criteria after three cycles. Three weeks after the completion of the last cycle, patients were taken up for surgery (MRM). All patients received adjuvant chemotherapy and radiotherapy based on their disease status.

Details of immunohistochemical assessment

The antibodies used included anti-CEACAM5 antibody (ab131070) (Rabbit polyclonal), m/s Abcam, anti-CEACAM6 antibody (ab56234) (Rabbit polyclonal), and anti-SLC7A5 antibody (ab85226), (Rabbit polyclonal), m/s Abcam. Immunohistochemical staining for CEACAM5 and CEACAM6 was defined as positive when cytoplasm and/or membrane staining was present on >10% of invasive tumor cells. For SLC7A5 positivity, plasma membrane staining on >10% of invasive tumor cells was taken as a criterion. Staining was further scored into four groups: (0) Indicated negative staining. (1) Indicated scattered cells weakly positive. (2) Indicated most cells weakly to moderately positive. (3) Indicated all cells strongly positive.

Statistical analysis of biomarkers - McNemar's Chi-square test and Student's paired *t*-test were used to determine the association between two variables. $P \leq 0.05$ was taken as significant. Data analysis was performed by SPSS 18.0 version, Chicago, IL.

RESULTS

A total of 30 histopathologically proven cases of carcinoma breast who were fit to receive NACT were included in the study. All the cases were staged as LABC based on American Joint Cancer Committee criteria. Baseline clinical features are depicted in Table 1. The mean age of the patients was 51.5 ± 11.1 years (range - 32–72 years). Sixty percent patients were postmenopausal, and only one patient had a positive family history.

The changes in tumor size in response to NACT are shown in Table 2. The mean size reduced from 6.3 ± 2.3 cm to 4.3 ± 2.6 cm. This change was statistically highly significant ($P < 0.001$).

Response to chemotherapy, as assessed by RECIST criteria, is shown in Table 3. 12/30 (40%) patients showed a positive response while in 3/30 (10%) patients, the disease continued to progress. Among those with a positive clinical response, four patients had complete clinical response. The results of immunohistochemical markers, viz; CEACAM5 [Figure 1], CEACAM6 [Figure 2], SLC7A5 [Figure 3], as related to response to NACT are shown in Tables 4-6. It was observed that CEACAM 5 level correlated well with response to NACT and this relation was found to be significant ($P = 0.004$). CEACAM 6 level also significantly correlated well with response to NACT ($P = 0.020$). Furthermore, relationship between response to NACT and node-positive tumors with SLC7A5 immunoreactivity was found to be highly significant ($P = 0.009$).

Table 1: Baseline clinical features

Age	Age groups	Number of patients	Percentage (*n*=30)
	31–40	7	23.3
	41–50	8	26.7
	51–60	9	30.0
	>60	6	20.0
MP status	Pre-MP	12	40.0
	Post-MP	18	60.0
Family history	Present	1	3
	Absent	29	97

MP: Menopausal

Table 2: Change in tumor size

Pre-NACT tumor size (cm)			Post-NACT tumor size (cm)		
<2	-	-	<2	5	16.7%
2.01-5.00	11	36.7%	2.01-5.00	14	46.7%
>5.00	19	63.3%	>5.00	11	36.7%
Mean size	6.3±2.3		Mean size	4.3±2.6	

NACT: Neo-adjuvant chemotherapy

Table 3: Assessment of response

Response	Number of patients	Percentage
PD	3	10
SD	15	50
PR	8	26.7
CR	4	13.3

PD: Progressive disease, SD: Stable disease, PR: Partial response, CR: Complete response

DISCUSSION

In India, breast cancer is the most common cancer among women in urban areas, and its incidence is still rising. LABC is the most common stage of presentation. NACT is a preferred modality of treatment in these patients. Potential advantages of administering NACT are manifold. Foremost among them is the longer disease free survival (DFS). In studies comparing NACT to adjuvant chemotherapy, pathological complete response (pCR) is directly associated with increased DFS and overall survivals.[17] Thus, the primary aim of any NACT now is to achieve a pCR. NACT allows the assessment of tumor response and analyses of prognostic variables to suggest a positive correlation between response and survival. With different approaches

Table 4: CEACAM5 expression in the study group

	CEACAM5				
Response	Score (%)				
	0	1	2	3	Total
PD	0	0	0	3 (13.6)	3 (10.0)
SD	0	1 (100.0)	2 (100.0)	12 (54.5)	15 (50.0)
PR	1 (20.0)	0	0	7 (31.8)	8 (26.7)
CR	4 (80.0)	0	0	0	4 (13.3)
Total	5 (100.0)	1 (100.0)	2 (100.0)	22 (100.0)	30 (100.0)

χ^2=26.284, *P*=0.004 (S). PD: Progressive disease, SD: Stable disease, PR: Partial response, CR: Complete response, CEACAM5: Carcinoembryonic antigen-related cell adhesion molecules 5

Table 5: CEACAM6 expression in the study group

	CEACAM6				
Response	Score (%)				
	0	1	2	3	Total
PD	0	0	2 (16.7)	1 (50)	3 (10.0)
SD	2 (22.2)	4 (57.1)	8 (66.7)	1 (50)	15 (50.0)
PR	3 (33.3)	3 (42.9)	2 (16.7)	0	8 (26.7)
CR	4 (44.4)	0	0	0	4 (13.3)
Total	9 (100.0)	7 (100.0)	12 (100.0)	2 (100.0)	30 (100.0)

χ^2=19.627, *P*<0.05(S) (0.020). PD: Progressive disease, SD: Stable disease, PR: Partial response, CR: Complete response, CEACAM6: Carcinoembryonic antigen-related cell adhesion molecules 6

Table 6: SLC7A5 expression in the study group

	SLC7A5				
Response	Score (%)				
	0	1	2	3	Total
PD	0	0	2 (22.2%)	1 (33.3)	3 (10)
SD	2 (20)	7 (87.5)	4 (44.4)	2 (66.7)	15 (50)
PR	4 (40)	1 (12.5)	3 (33.3)	0	8 (26.7)
CR	4 (40)	0	0	0	4 (13.3)
Total	10 (100)	8 (100)	9 (100)	3 (100)	30 (100)

χ^2=21.836, *P*<0.05(S) (0.009). PD: Progressive disease, SD: Stable disease, PR: Partial response, CR: Complete response

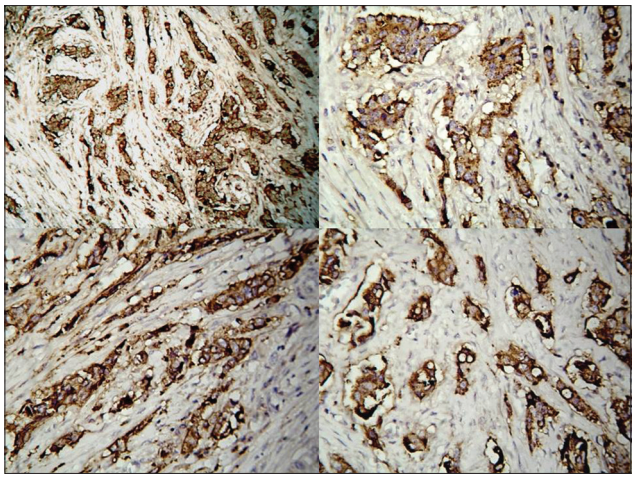

Figure 1: Photomicrograph showing membrane and cytoplasmic immunoreactivity for carcinoembryonic antigen-related cell adhesion molecules 5 antibody (abcam) in infiltrating duct carcinoma breast

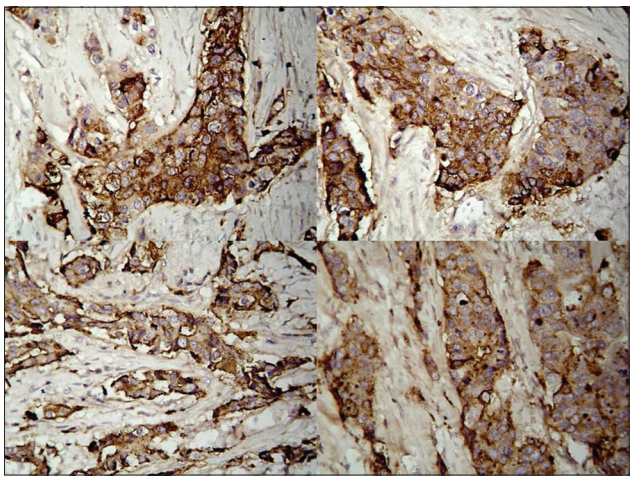

Figure 2: Photomicrograph showing membrane and cytoplasmic immunoreactivity for carcinoembryonic antigen-related cell adhesion molecules 6 antibody (abcam) in infiltrating duct carcinoma breast

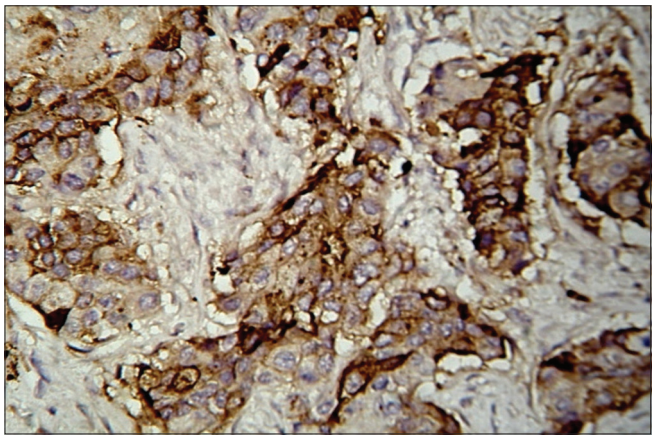

Figure 3: Photomicrograph showing membrane immunoreactivity for SLC7A5 antibody (abcam) in infiltrating duct carcinoma breast

to modulating neo-adjuvant therapy, there is a trend toward greater emphasis on response benefit than on survival. Theoretically, remaining primary or drug induced resistant cells could be present even in highly responsive tumors and affect long-term outcome. Cellular drug resistance mechanism need further study, preferably with repeated analyses to understand the changes of phenotype over time in tumor evolution from local to systemic disease. Resistance to chemotherapy is a pertinent problem and so identification of reliable biomarkers that can predict response to chemotherapy is an important lacuna that needs to be filled.

The human carcinoembryonic antigen (CEA) family has seven genes belonging to the CEACAM subgroup. These subgroup members are mainly associated with the cell adhesion, migration and invasion. The cell adhesion molecules of CEA attach with cell membrane and have complex regulatory function of cell adhesion and tumor cell chemosensitivity.[18,19] These molecules have differential expression in normal and cancerous tissues. Levels of expression have important bearing in the determination of response to chemotherapy.[18] CEACAM5 and CEACAM 6 are important molecules of this category. CEACAM5 expression is supposed to be a means for overcoming the apoptosis-inducing therapies.[14] Increased expression of both CEACAM5 and CEACAM6 inhibits apoptosis. Also, CEACAM6 over expression has been shown to independently predict poor overall survival and poor disease-free survival.[20] Ultimately targeting these molecules also serves as novel methods of modulating chemosensitivity and apoptosis. Antibodies against these drugs can act as chemosensitizers.[21]

In this study, 25 patients had detectable levels of CEACAM5. Twenty-two patients had higher levels (3+) of CEACAM5 in tumor cells. Five patients had undetectable levels of CEACAM5. Of these 5, 4 had pCR. Twelve patients (40%) had moderate levels (2+) of CEACAM6 and nine patients had undetectable levels of CEACAM6.

A significant NEGATIVE relationship between CEACAM5 and CEACAM6 and response to NACT was found ($P = 0.004$ and $P = 0.020$, respectively). Association of CEACAM5 and CEACAM6 with breast cancer appears to be reasonably well-established. The antibodies to these are an area of on-going research; however, their dynamics with the therapy is a relatively unexplored area. Duxbury *et al.* have shown that silencing CEACAM6 by siRNA: (a) Enhances cell anoikis, (b) increases caspase activation in response to anchorage-independent conditions, (c) down-regulates the Akt cell survival pathway, (d) inhibits metastasis *in vivo*, and (e) enhances gemcitabine induced chemosensitivity.[22] Recent reports suggest that CEACAM6 targeted antibodies are excellent blockers of cancer progression[21] and vaccines based on CEACAM6 in clinical trials for preventing the

progression of breast cancer have been highly promising.[23] Targeting CEACAM5 and/or CEACAM6 may therefore be a novel method of modulating cancer cell chemosensitivity and apoptosis. We could not come across a study similar to ours, but our data appear to be in line with currently available facts. Hence, according to the results, low CEACAM5 and CEACAM6 levels may be used as a predictor for response to NACT in breast cancer.

Correlation of SLC7A5 with response to neo-adjuvant chemotherapy

SLC7A5 is part of a two-protein complex with SLC3A2, the heavy chain of a neutral amino acid transporter implicated in nutrient transport at the blood-brain barrier.[24,25] By gene expression pattern, it clusters with markers of proliferation in breast cancer and has been previously noted to be highly expressed in some cancers.[26,27] In this study, relationship between response to NACT with SLC7A5 immunoreactivity was found to be highly significant (P = 0.009). Hence, according to the results, SLC7A5 may be used as a predictor for response to NACT in breast cancer.

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