Original Article

Role of Tc99m-Sestamibi scintimammography in assessing response to neoadjuvant chemotherapy in patients with locally advanced breast cancer

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ABSTRACT

Introduction: Neoadjuvant chemotherapy (NACT) is an essential part of multi-disciplinary management of locally advanced breast cancer (LABC). In this study, we aimed at evaluating the role of Tc99m-Sestamibi scinti-mammography in assessing response to NACT in patients with LABC. **Materials and Methods:** A total of 42 patients of histologically proven LABC were enrolled in this prospective study. Imaging was performed according to pre-defined protocol at 10 min and 4 h after injection of tracer before the start of chemotherapy, 48 h after start of chemotherapy and at the end of chemotherapy. Quantitative parameters were obtained by calculating the ratio of activity in a region of interest (ROI) drawn over the tumor and the same sized ROI drawn in corresponding location in contra lateral breast. **Results:** At the end of chemotherapy, 6 patients achieved complete response, 25 achieved partial response, 11 had stable disease. Various retention indices calculated at baseline, 48 h after first cycle of NACT, and at the end of chemotherapy showed statistically significant difference in responders and non-responders. By using 84.05 as cut-off point for retention index (RI) of tumor calculated 48 h after first cycle of NACT (RI 2) the positive predictive value and negative predictive value, were found to be 41.9% and 72.7% respectively in differentiating responders from non-responders. **Conclusion:** Early response assessment in patients with LABC to NACT with Tc99m-Sestamibi scintimammography is feasible and if confirmed by further studies can find routine clinical application in differentiating responders from non-responders.

Keywords: Locally advanced breast cancer, response evaluation, scintimammography, Tc99m-Sestamibi

INTRODUCTION

Breast cancer is the second most common cancer of women in the developing world, the incidence being 22.9 per lac population in India in 2008.[1] Breast cancer is responsible for 12.1% of the cancer burden in women.[1] Locally advanced breast cancer (LABC) refers to primary tumor lesion larger than 5 cm in diameter or involving chest wall or skin or fixed axillary nodes (T3 or T4 with any N or N2 or N3 with any T, i.e., all patients with stage III and some with stage IIB, as per American joint committee on cancer (AJCC) tumor, nodes, metastasis (TNM) staging).[2] LABC has an incidence of 10-20% in the developed world and as high as about 30-60% in the developing world.[3] These tumors have a high relapse rate and mortality.

The current approach of initial treatment of LABC is multi-disciplinary and includes neoadjuvant chemotherapy (NACT), surgery, radiotherapy and adjuvant chemotherapy with or without hormone therapy. The initial treatment is NACT to provide better local disease control.[4] The optimal duration of NACT remain controversial, in part because of difficulty in evaluating response to therapy. In these patients appropriate and early response assessment is critical for planning further management.

The response is conventionally assessed clinically (size), radiologically (ultrasound, contrast enhanced computed tomography, magnetic resonance imaging and mammography), and by pathological analysis of the resected specimen. The main disadvantage of these methods is that response can be assessed

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only after completion of the chemotherapy. Hence, none of these methods can be used in the early phase of the chemotherapy. Another issue with the size or image based response evaluation is the fact, that tumor killing is accompanied by fibrosis, which causes a mass lesion, thus, making the size criterion unreliable to assess tumor response. Techniques that measure changes in tumor biology rather than anatomical changes, and hence can be used in early phase of treatment, like functional magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging need to be evaluated for this purpose.

Scintimammography using Tc99m-Sestamibi is an accepted non-invasive diagnostic nuclear imaging SPECT technique that can also be used for response assessment in patients with breast cancer.[5] Scintimammography using Tc99m-Sestamibi is a more affordable and easily available modality as compared to other functional imaging alternatives, like MRI and PET. Tc99m-Sestamibi is a lipophilic SPECT tracer which concentrates in the cancer cells by an energy requiring transport mechanism and a transmembrane electronegative potential, apart from other non-specific mechanisms. It is stored inside the mitochondria.[5] It concentrates preferentially in breast tumor cells as compared to normal breast tissue, increased blood flow to tumor cells being one of the possible causes for this preferential behavior.[6,7] Tc99m-Sestamibi is also a potentially useful marker of response to NACT, reduction of uptake post-chemotherapy (as compared to that before chemotherapy) indicating favorable response to the given chemotherapy.[8-10]

The aim of this study is to assess the changes in 99m-Tc-Sestamibi uptake within the tumor induced by NACT and to correlate these changes to the response at the end of therapy in patients with LABC.

MATERIALS AND METHODS

A prospective study was carried out on female patients with histologically proven LABC (as per AJCC TNM staging[2] who were planned to receive NACT. Patients with previous breast surgery, chemotherapy, hormone therapy, radiotherapy or any co-morbities rendering the patient ineligible for surgery, were excluded from the study. All patients underwent a complete clinical examination. All patients were treated with four courses of anthracycline based NACT: FAC (5-Fluorouracil – 500 mg/m^2 IV, Adriamycin – 50 mg/m^2 IV, Cyclophosphamide – 500 mg/m^2 IV) or < FEC (5-Fluorouracil – 500 mg/m^2 IV, Epirubicin – 100 mg/m^2 IV, Cyclophosphamide – 500 mg/m^2 IV). The cycles were repeated every 3 weeks. Patients were monitored with liver function tests, renal function tests and complete blood counts before each cycle. The response to chemotherapy was assessed using WHO criteria,[11] at the end of 1st cycle and after four cycles of NACT. The patients were grouped as complete responders (CR), partial responders (PR), stable disease (SD) and progressive disease (PD) according to their response to NACT. Scintigraphic studies were performed before starting chemotherapy, 48-72 h after the first dose of chemotherapy, and after completion of four cycles of the NACT. 20 mCi of 99m-Tc-Sestamibi was administered intravenously in the dorsal foot veins (preferably), or in an antecubital vein of the arm contralateral to the side of tumor. Ten minutes later, breast imaging was performed, first of the involved beast followed by the normal breast, in a 256 × 256 matrix using a gamma camera with a high resolution general purpose collimator, in the anterior projection followed by lateral projection in the prone position with a shielding pallet between the two breasts. A lateral prone imaging of the affected and the contralateral normal breast was then acquired 4 h post-injection, keeping all the imaging parameters same as that in the earlier image. Data acquired was stored in an online computer for subsequent evaluation.

All the data was first qualitatively evaluated and site of abnormal tracer uptake was ascertained in the tumor lesion in the affected breasts. Thereafter, semi quantitative analysis was done for both early and delayed images, by drawing a region of interest (ROI) over the abnormal tracer uptake region at the tumor site in the affected breast and a similar ROI was also drawn on the normal contralateral breast at the same site as that in the affected breast. Uptake ratio was calculated for each tumor lesion in both the early and delayed images, by dividing the counts obtained in the ROI over the lesion by the counts in the ROI over the same region in the contralateral normal breast. Tumor retention index (RI) was calculated by dividing the delayed uptake ratio – lesion uptake ratio at 4 h, by the early uptake ratio – lesion uptake ratio at 10 min. All the ROI measurements were done by the same observer. A fall in the RI at completion of four cycles of NACT was considered as favorable response whereas no significant fall was considered as resistant to the given NACT. Difference in retention indices in between groups in pre- and post-NACT was analyzed by ANOVA statistical test. $P < 0.05$ was considered as statistically significant.

RESULTS

A total of 42 patients were enrolled in the study and evaluated. Their mean age was 41.68 years (range from 30 years to 55 years). Thirty two women were pre-menopausal.

Primary tumor parameters

The tumor size ranged from 5 cm to 10 cm (mean size 6.40 cm ± 0.99 cm). According to TNM stage groupings, the distribution in different stages was as follows: 24 (57.14%) in stage IIIA, 14 (33.33%) in stage IIIB, and 4 (9.52%) in stage IIIC [Figure 1]. Thirty nine patients had ductal carcinoma while three patients had lobular carcinoma.

Chemotherapy regimens

FEC regime was administered to 14 patients (33.33%) and 28 patients (66.66%) received FAC regime. Following NACT, grade I and II nausea and vomiting was present in 15 cases (35.71%) but none had grade III or grade IV toxicity. All patients received four cycles of chemotherapy. Six patients achieved complete response (CR), 25 achieved partial response (PR),

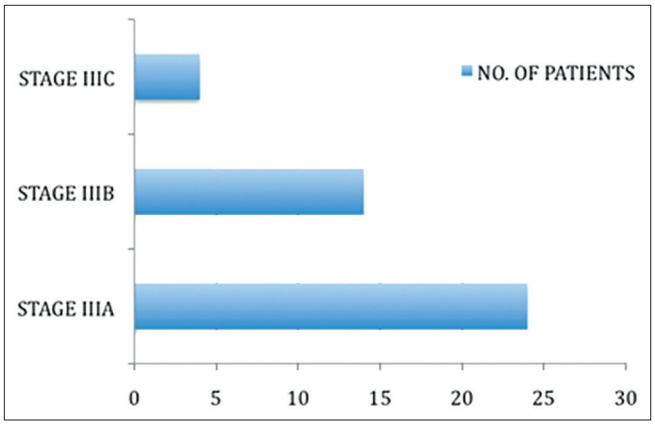

Figure 1: Stage wise distribution of patients enrolled in the study

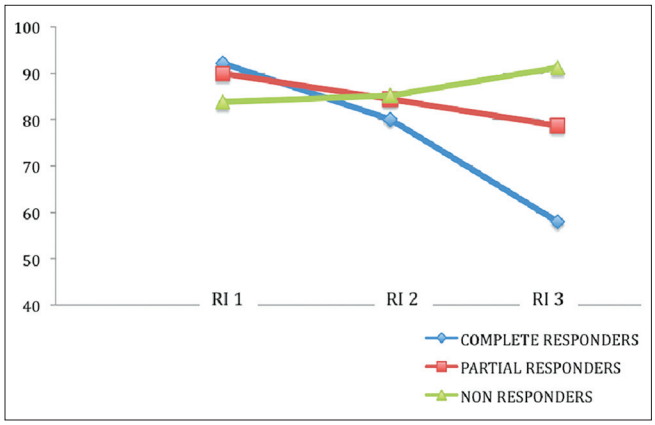

Figure 2: Change in retention index with neoadjuvant chemotherapy

and 11 had SD at the end of chemotherapy. For statistical analysis patients were grouped into responders (CR + PR) and non-responders (SD). Mean tumor size in complete responders prior to NACT was 5.91 cm ± 0.49 cm (range: 5.5-6.5). Mean tumor size in partial responders was 6.52 cm ± 1.08 cm (range: 5-10 cm) prior to NACT and was 2.85 cm ± 0.45 cm (2-4 cm) post-NACT. In case of non-responders tumor size was 6.55 cm ± 1.01 cm (range: 5.5-8.5 cm) prior to NACT and was 5.68 cm ± 0.87 cm (4.5-7.0 cm) post-NACT

RI

The values of tumor retention index (RI) before starting NACT (RI1), after 48 h of 1st cycle of NACT (RI 2) and after completion of the 4th cycle of NACT (RI 3), in complete responders were 92.1 ± 8.2, 80 ± 7 and 58 ± 28.9 respectively. RI1, RI2 and RI3 were 89.9 ± 9.6, 84.4 ± 8.3, 78.7 ± 8.5 respectively in case of partial responders, whereas in case non-responders the values of RI1, RI2 and RI3 were 83.8 ± 13.3, 85.2 ± 10.6 and 91.2 ± 14.7. The change (increase) in mean RI in non-responders was statistically significant between RI3 and RI2. Whereas in partial and complete responders the change (decrease) was significant between – RI2, RI1; RI3, RI1 and RI3, RI2 for clinical application, using RI2 = 84.05 as cut off point for classifying patients into responders and non-responders, the sensitivity was found to be 81.25%, specificity, 30.7%, positive predictive value (PPV), 41.9 and NPV, 72.7. Figure 2 represents change in RI with NACT.

Receiver operating characteristic (ROC) curve analysis was performed to calculate a clinically significant value of RI 2 by which response groups can be predicted and the value for RI 2 was 84.05. By using this threshold as cut-off, PPV and NPV were found to be 41.9% and 72.7% respectively in differentiating responders from non-responders. All the results are summarized in Tables 1-4.

Table 1: Response rate to two different types of chemotherapy regimens

Response rate	FAC (*n*=28)	FEC (*n*=14)	Total (*n*=42)
Complete response (%)	3 (10.7)	3 (21.4)	6 (14.3)
Partial response (%)	19 (67.9)	6 (42.9)	25 (59.5)
No response (%)	6 (21.4)	5 (35.7)	11 (26.2)
Progressive disease (%)	0	0	0

FAC: Fluorouracil adriamycin cyclophosphamide , FEC: Fluorouracil epirubicin cyclophosphamide

Table 2: Change in size before and after chemotherapy

Tumor size (cm)	Pre-NACT	Post-NACT
Complete response	5.91±0.49 (5.5-6.5)	Not measureable
Partial response	6.52±1.08 (5-10)	2.85±0.45 (2-4)
No response	6.55±1.01 (5.5-8.5)	5.68±0.87 (4.5-7)

NACT: Neoadjuvant chemotherapy

Table 3: Retention indices at different time points during the study

Response group	RI 1	RI 2	RI 3
Complete responders	92.1±8.2	80±7	58±28.9
Partial responders	89.9±9.6	84.4±8.3	78.7±8.5
Non responders	83.8±13.3	85.2±10.6	91.2±14.7
All patients	88.6±10.7	84.05±8.8	79±17.3

RI 1: Retention index in base line study, RI 2: Retention index 48 h after first cycle of NACT, RI 3: Retention index after 4 cycles of NACT, NACT: Neoadjuvant chemotherapy

Table 4: Performance of retention index 48 h after first cycle of neoadjuvant chemotherapy in predicting the response

Response group	No. of cases with RI 2<84.05	No. of cases with RI 2>84.05
Responders (partial and complete)	13 (True positive)	18 (False positive)
Non responders	3 (False negative)	8 (True negative)

RI: Retention index

DISCUSSION

Neoadjuvant or pre-surgical chemotherapy has become the standard of care for patients with LABC. It provides early treatment of micrometastases and causes size reduction of the tumor in patients in whom it is otherwise may be difficult to obtain adequate surgical margins. It can also change the management in some cases from mastectomy to lumpectomy. It also provides an *in vivo* indication of tumor sensitivity to chemotherapy drugs in patients who ultimately will require post-surgical adjuvant chemotherapy.[12-15] However, it must be emphasized that not all patients with LABC respond to

NACT. Hence, it is important to monitor the NACT and change the dose and/or the regimen as and when required providing an effective response in appropriate patients and lessening the window period in changing to other forms of therapy. It is therefore logical to question whether adjusting the pre-surgical treatment schedule to achieve maximal response will improve the outcome in patients with LABC. This would require individualized therapy based on patient response. Unfortunately, the limitations of the conventional methods for response assessment make it difficult to assess the quantity of residual viable tumor during the course of NACT accurately. A method which could predict response to NACT earlier in the course of therapy would result in the ability to treat effectively to maximal response and help in planning the optimal timing of surgery.

Role of scintimammography in the diagnosis of breast cancer is well established by many studies until date.[16-19] As mentioned earlier 99m-Tc-Sestamibi concentrates in cancer cells by an energy dependent transport mechanism and a transmembrane electronegative potential, in addition to other non-specific mechanisms. The tracer uptake within the cancer cells can be quantitatively assessed. The uptake is directly proportional to the active transport mechanisms in the cancer cells (i.e., quantity of functionally active cells).

The rationale behind using scintimammography (using Tc99m-Sestamibi) for response assessment to NACT is that, effective chemotherapy kills the cancer cells, thus reducing the number of functionally active cancer cells, which in turn reduces the uptake of 99m-Tc-Sestamibi within the tumor mass, causing reduction in the tracer retention within the tumor post-effective chemotherapy as compared to that before starting the chemotherapy. Many studies have been published emphasizing the role of Tc-99m-sestamibi in response assessment to NACT in patients with breast cancer.[8,20,21-24]

This study, though based on same physiologic mechanisms as used by the above mentioned studies, is able to detect responders at a very early stage of NACT (48 h after the first cycle) without requiring any other special investigations (immunohistochemistry etc.), thereby differentiating it from the other studies. Mankoff *et al*.[8] in their study had assessed response to NACT after 2 months from the initiation of therapy and Mezi *et al*.[20] after completion of NACT. Takamura *et al*.[24] did suggest a methodology for early response assessment; however, it required immunohistochemistry for determining P-glycoprotein 1 (Pgp) expression and polymerase chain reaction for determining mRNA (multidrug resistance protein 1 [MDR 1]) expression, which made the entire process of response assessment cumbersome and expensive.

A point of concern in many of these studies was whether to use early or delayed uptake for response assessment, since early uptake was affected by tumor blood flow. However, in this study, this issue was resolved by using RI, which is the ratio of the delayed uptake to early uptake, for response assessment rather than individual (early or delayed) uptake values.

In this study, another aspect of mechanism of Tc-99m-Sestamibi uptake was exploited. As stated earlier, increased Pgp expression in the tumor cells results in lesser retention of Tc-99m-Sestamibi within the tumor cell, which in turn reflects lesser probability of the tumor to respond to the given therapy since Pgp expression is encoded by the MDR (multi-drug resistance) gene. The expression of MDR gene predicts increased probability of resistance to the given therapy. Our study highlights this, by showing higher mean value of RI in responders (92.12), as compared to that in partial responders (89.93) and lowest being in non-responders (83.82).

The decrease in RI 2 and RI 3 was significant in both complete and partial responders thus indicating that responders (complete or partial) can be identified at an early stage of NACT. This was not seen in non-responders, instead it was noted that RI in these continued to increase or remained same up till the end of four cycles of NACT. This correlates well with the study conducted by Tiling *et al*.[23]

CONCLUSION

Based on this study, we can conclude that decrease in RI before and after first cycle of chemotherapy (RI1 – RI2) is significant in case of complete and partial responders, and can be used for early response assessment. It can also be concluded that the reduction in RI with effective chemotherapy correlates and reflects effective cell killing. Furthermore, decrease in RI before and after first cycle of chemotherapy (RI1 – RI2) is not significant in case of non-responders. Also, scintimammography is a safe and affordable investigation, which can be used in early detection of those groups of patients with LABC, in whom NACT is effective.

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