STUDY OF METASTATIC CANCER OF BREAST WITH SPECIAL REFERENCE TO BREAST CANCER SUBTYPES

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ABSTRACT

Background: Breast cancer is a major medical problem with significant public health and societal ramifications and is a leading cause of cancer death in women. Gene expression profiling has identified five subtypes of breast cancer (luminal A, luminal B, normal breast like, HER-2neu over expression, and basal-like), each of which has a different prognosis. **Aims & Objectives:** The aim of the present study to compare the expression of these biomarkers between primary and metastatic breast carcinoma. **Material & Methods:** All female patients ages >18 years diagnosed with metastatic breast cancer at the Gujarat Cancer and Research Institute from October 2010 to February 2013 were evaluated and taken into the study. The assessment of ER, PR and Her2 for the metastatic tissue was then compared with that of the primary tumour. **Results:** In our study the youngest patient was 25 years old and the eldest was 75 years old with an average age and median age was 45 years at the time of presentation with breast cancer. Estrogen receptor (ER) was positive in 15 (23%) and negative in 50 (77%) primary tumours while positive in 27 (42%) and negative in 38 (58%) metastatic tumours. Progesterone receptor (PR) was positive in 20 (31%) and negative in 45 (69%) primary tumours while positive in 24 (37%) and negative in 41 (63%) metastatic tumours. HER2 receptor was positive in 25 (38%) and negative in 40 (62%) primary tumours while positive in 22 (34%) and negative in 43 (66%) metastatic tumours. **Conclusion:** Our study demonstrates important differences in metastatic behaviour between the breast cancer subtypes as defined by a panel of immunohistochemical markers and contributes to an expanding knowledge of prognostic and predictive markers that will allow individualized therapy for metastatic breast cancer.

Key-words: Breast Cancer, Estrogen receptor, Progesterone receptor, HER2 receptor

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INTRODUCTION

Breast cancer is the most common female cancer worldwide. Although its incidence appears to be levelling off in Western countries, after decades of increasing, it is still high and continues to increase in certain countries where it initially had low incidence.[1] It is a heterogeneous disease with regard to biological behaviour, responses to treatment and prognosis.[2,3] Therefore, further understanding of the biology of the disease is needed to improve treatment outcome and reduce mortality.[2] Gene expression profiling has identified five subtypes of breast cancer (luminal A, luminal B, normal breast like, HER-2neu over expression, and basal-like), each of which has a different prognosis.[4–6] The basal-like and HER-2neu+ subtypes have shorter relapse-free survival (RFS) and overall survival (OS) than the luminal tumors.[4, 7] Despite significant advances in the diagnosis and treatment of breast cancer, approximately one-third of patients still develop and subsequently die from metastatic breast disease. The range is very wide, however, with some patients having more indolent disease that they can live with for 10–15 years, while for others with widespread metastatic disease, the prognosis may only be a matter of months from the time of diagnosis.[8]

Bone is the most common metastatic site in all subtypes except basal-like tumours. In multivariate analysis, compared with luminal a tumours, luminal/HER2 and HER2-enriched tumours were associated with a significantly higher rate of brain, liver, and lung metastases. Basal-like tumours had a higher rate of brain, lung, and distant nodal metastases, but a significantly lower rate of liver and bone metastases.[9] There is a slight decrease in expression of these bio-markers in the metastatic tumours. This effect may be due to tumour heterogeneity, a well-known fact, in anticancer chemo sensitivity, and may be reflected in hormonal receptor status of metastatic breast carcinoma. Neoplastic cells from high-grade tumours may also loose estrogen and progesterone receptors during the process of metastasis. HER-2/neu expression, however, remains almost same in primary and metastatic breast carcinomas.[10,11] Metastatic breast cancer is an important area of research for both researchers and clinicians because MBC has a poor prognosis. The present study was conducted to compare the expression of these bio-markers between primary and metastatic breast carcinoma.

MATERIAL & METHODS

The present prospective study was conducted in the Department of Medical Oncology at the Gujarat Cancer and Research Institute, Ahmadabad after taking the permission from the Institutional Research Committee. All the female patients age >18 years diagnosed with metastatic Breast Cancer at The Gujarat Cancer and Research Institute from October 2010 to February 2013 were evaluated and taken into the study after taking consent. At baseline, all patients underwent a detailed history and physical examination. The information abstracted included demographic data; present complaints, vital statistics; menopausal status, family history of cancer, other medical conditions, receptor status, sites of metastases and previous treatment received.

Selection of the patients: All female Patients with recent Metastatic Breast Carcinoma (MBC), synchronous or metachronous and not started treatment for MBC; having ER, PR and Her2neu status of Primary breast cancer known were included in the present study. All breast cancer patients with non-epithelial origins, male breast cancer, patients with unknown receptor status (ER/PR/HER2) of primary tumour in case of metachronous breast cancer, were excluded.

Complete *history of primary breast cancer,* including date and type of surgery, side, histopthological details (tumour size, histology, grade, margins, lympho-vascular invasion and lymph node status), stage, receptor status, USG abdomen pelvis, CXR, mammogram, bone scan and treatment (chemotherapy/radiotherapy) received were noted. Distant relapse was defined as recurrences of breast cancer occurring beyond the confines of the ipsilateral breast, chest wall, or regional lymph nodes. Sites of distant relapse were categorized as follows: brain (including choroid, CNS, pituitary gland, leptomeningeal, and frontal sinus), liver, lung (including lymphangitic carcinomatosis), bone (including bone marrow), distant nodal (nodes beyond the ipsilateral axillary/supraclavicular/internal mammary area), pleural/peritoneal (including ascites, omentum, pleural effusion, and peritoneal carcinomatosis), and other (including skin outside of breast/chest wall, ovaries, spinal cord, eye, heart, and other organs not elsewhere classified). According to primary breast cancer subtype, metastatic sites were correlated and the assessment of ER, PR and Her2 for the metastatic tissue was then compared with that for the primary tumour. Information about adjuvant therapy received by each patient and time between initial diagnosis and metastatic presentation were also documented.

Biopsy Procedures: For superficial metastasis core, punch or excisional biopsy was performed using palpation guidance only. For internal lesions, the most feasible site of biopsy was determined in consultation with an interventional radiologist, and FNA, core biopsy, or aspiration of fluid (pleural, ascetic, CSF) was carried out under radiologic guidance. When aspiration was undertaken, smears of tumour cells and cell blocks were prepared. Samples were fixed in10% formalin within 20 minutes of the biopsy and processed using the same protocol as in other tissues (including formalin fixation for ≥8 hours). To optimize analysis of receptor expression, biopsies of metastatic bone lesions were not decalcified whenever possible.

Tissue Processing: All biopsies were evaluated by expert pathologist and/or cytopathologist. Confirmation of malignancy and evaluation of hormone receptor and HER2 expression were analyzed from all available samples. Primary tumour tissues in which previously ER, PR and/or Her-2 neu were not done, this analysis was performed.

Immunohistochemistry:

Immunohistochemical staining was performed by the IHC division of pathology department for Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2 on the biopsy specimen from metastatic site with Ventana Benchmark XT autostainer using ultra view DAB detection kit. ER positivity and

PR positivity were defined as any positive nuclear staining (i.e., ≥1%).

According to ASCO/CAP guidelines, HER-2 expression was scored as 0 (no staining or weak/moderate, incomplete/ complete staining in ≤10% of cells), 1+ (weak and incomplete staining in >10% of cells), 2+ (weak/moderate complete staining in >10% of cells or strong, complete staining in ≤30% of cells) and 3+ (strong, complete staining in >30% of cells). FISH testing was not done for Her-2 neu 1+ or 2+ score because of no availability at our institute.

Breast cancer molecular subtypes are classified according to a gene expression profile–validated immunohistochemical surrogate panel[26-28] as follows: luminal A (ER positive and/or PR positive), luminal B (ER positive and/or PR positive), luminal/HER2 (ER positive and/or PR positive and HER2 positive), HER2 enriched (ER negative and PR negative and HER2 positive), and basal-like (ER negative and PR negative and HER2 negative). But as we have analyzed receptor status with IHC only so subtypes grouped as:

1. Hormone Receptor (HR) Positive i.e. ER/PR + and Her2neu –
2. Triple Positive i.e. ER/PR + and Her2neu +
3. HER-2 Positive i.e. ER/PR - and Her2neu +
4. Triple Negative/Basal i.e. ER/PR - and Her2neu –

RESULTS

One hundred and twenty cases (n=120) of metastatic breast cancer patients were enrolled in the present study from October 2010 to February 2013. Out of 120 patients, 95 patients presented with metachronous metastases and 25 presented with synchronous metastases. All patients underwent evaluation at the time of metastasis. In our study youngest patient was 25 years old and the eldest was 75 year old with average age and median age was 45 years at the time of presentation with breast cancer. Hormonal receptor status was compared between primary and metastatic tumours (table 2). Estrogen receptor (ER) was positive in 15 (23%) and negative in 50 (77%) primary tumours while positive in 27 (42%) and negative in 38 (58%) metastatic tumours (table 1). Progesterone receptor (PR) was positive in 20 (31%) and negative in 45 (69%) primary tumours while positive in 24 (37%) and negative in 41 (63%) metastatic tumours. HER2 receptor was positive in 25 (38%) and negative in 40 (62%) primary tumours while positive in 22 (34%) and negative in 43 (66%) metastatic tumours (table 2 & Graph 1).

The results regarding ER, PR and HER-2 status in the primary tumor and corresponding metastatic sites are shown in the above table. A change in ER was present in 20 (30.7%) samples, four (26.7%) lost and sixteen (32%) gained ER expression. A change in PR status was present in 28 (43%) samples; 12 (60%) had a loss and 16 (35.5%) had gained of PR expression. Change in HER-2 status was observed in 13 of 65 samples (20%); 8 (32%) had a loss and 5 had (12.5%) gain of expression (table 3).

DISCUSSION

Despite improvements in screening and treatment, breast cancer remains the most common cause of cancer and the second-

leading cause of cancer-related death in women. It is expected that 1 in 8 women will develop breast cancer in their lifetime.[12,13] Most patients present with an early stage of breast cancer, but 6% to 10% of patients initially present with metastatic breast cancer (MBC), defined as cancer occurring at sites distant from the breast, chest wall, and regional lymph nodes (mainly bone, lung, liver, and brain).[14] However, most patients who present with MBC have a recurrence of early-stage breast cancer.[15,16] One hundred and twenty cases (n=120) of metastatic breast cancer patients were enrolled in the study from October 2010 to February 2013. Out of 120 patients 95 presented with metachronous MBC and 25 presented with synchronous MBC. The median survival for MBC is approximately 18 to 30 months, so therapeutic decisions should be realistic and patient-specific.[14-16] Except for rare cases, MBC is not currently considered curable; thus, therapy primarily focuses on prolonging survival, maintaining quality of life, and delaying disease progression. Many factors must be considered when choosing a treatment pathway for MBC. Assessment of tumour biology, hormonal estrogen-receptor (ER) and progesterone-receptor (PR) status, as well as human epidermal growth factor receptor ([EGFR] also known as ErbB2, HER2, or HER2/neu) over-expression, are important determinants guiding therapeutic choices.[15,16]

In the present study, the median age at presentation was 45 years (range 25 to 75 years) which is comparable to 45 years (range 23-84 yrs) reported in the study by Muhammad Azam et al[17] but lower than what reported by Kennecke H et al[9] (56 yrs, range 42-71 yrs and Giuseppe Bogina et al[18] (61.2 yrs, range 34-93 yrs). In our study, median age was 41 yrs, 50 yrs, 42 yrs and 46 yrs in Hormone receptor positive, triple positive, HER2 positive and triple negative subtypes, respectively, while it was 62 yrs, 58 yrs, 56 yrs and 56 yrs respectively in study by Kennecke H et al.[9]

The average age of the high risk group in India is 43-46 years, unlike in the west where women aged 53-57 years are more prone to breast cancer. So average age of presentation with breast cancer is around 10 years younger in India as compared to the developed world and is the result of the age structure of the Indian population which is a bottom-heavy (predominantly young) pyramid.[19] In our study, significant discordances were found in the hormone receptor status between primary and metastatic breast pathology samples. A change in ER was present in 20 (30.7%) samples [four (26.7%) lost and sixteen (32%) gained ER expression]. A change in PR status was present in 28 (43%) samples [12 (60%) had a loss and 16 (35.5%) had a gain of PR expression]. Lower et al[20] found a discordance rate for ER of 30 % in a chart review of 200 patients with 19.5% of tumours losing ER and 10.5% gaining ER. For PR, this group found a discordance rate of 39.3%. Mobbs et al[21] performed a retrospective pathology specimen review of 129 cases and found discordance rates of 24 and 30% for ER and PR respectively. Gross et al,[22] in their series of 161 cases, found that 44% of patients lost PR, however 8% of patients gained PR. And finally a meta-analysis of 8 observational studies was performed by Franco et al[23] totalling 658 paired ER samples and 418 paired PR samples. They found a discordance rate of 29 and 27% for

ER and PR respectively. Reuben J Broom[24] in their 100 cases, found 17.7% change in ER and 37.3% in PR.

There are less data available on changes in Her-2/neu status with time, however, all the series published to date suggest that Her-2/neu status is more stable. In present study change in HER-2 status was observed in 13 of 65 samples (20%); 8 (32%) had a loss and 5 had (12.5%) a gain of expression.

Discordant receptor results can be caused by factors like:

(a) A genuine switch in the biology of the disease,
(b) Intratumor heterogeneity (i.e., sampling error),
(c) Clonal selection,
(d) Variable ER-lineage differentiation of a putative disseminated breast cancer stem cell during the course of the disease, and
(e) Limited accuracy and reproducibility of receptor assays.

Technical variability: Variable staining results are a result of differences in tissue fixation, antigen retrieval, and staining methods. Subjective scoring of results also contributes to less than perfectinter observer reproducibility. False-negative rates for ER status (relative to a reference laboratory) could be as high as 60%, even when the same specimens are analyzed by different laboratories. The length of fixation can have a profound influence on ER positivity rates: some strongly ER+ tumors can become completely negative if the fixation time is reduced. Despite important quality control initiatives, ER and HER-2 determinations remain variable in routine clinical practice even today.[25]

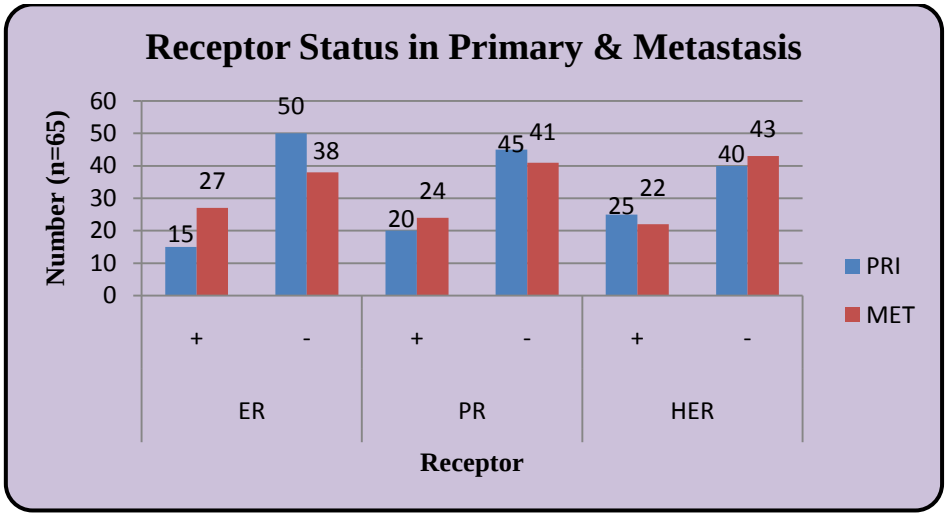
How will these changes in receptor status results impact everyday clinical practice? Certainly a change from a negative to a positive receptor status will impact management in that targeted therapy may then be incorporated into the treatment plan. Conversely, a change from a positive to a negative status would not only avoid the use of these agents and their related side effects but also would considerably cut down on unnecessary costs. Regarding therapeutic implications, change in clinical management in 7/20 (35%) patients with changes in ER status and 5/28 (17.8%) patients with change in PR status was noticed in our study. Amir et al,[26] Locatelli et al[27] and Thompson et al[28] reported a therapeutic change of management in 15.1%, 12.1% and 17.5%, respectively. Simmons et al[29] demonstrated a change in patient management in 6 of 29 cases (20%).

Table no. 1: Age Distribution of Study Population at the Time of Diagnosis of Breast Cancer (n=120)

AGE RANGE IN YEARS	NO. OF PATIENTS (%)
18-30	8 (6.7%)
31-40	36 (30.0%)
41-50	39 (32.5%)
51-60	30 (25%)
>60	7 (5.8%)
Total	**120**

Table 2: Comparison of Receptor Status Between Primary & Metastatic Tumors

RECEPTORS	PRIMARY BREAST TUMOR	METASTATIC TUMOR	P VALUE
Total no. of Patients	65 (100%)	65 (100%)	
Estrogen Receptors (ER)			
Positive	15 (23%)	27 (42%)	
Negative	50 (77%)	38 (58%)	0.0244
Progesterone Receptors (PR)			
Positive	20 (31%)	24 (37%)	
Negative	45 (69%)	41 (63%)	0.4587
HER-2/neu			
Positive	25 (38%)	22 (34%)	
Negative	40 (62%)	43 (66%)	0.5839



Graph no. 1: Showing Receptor status in primary & metastasis

Table 3: Change in er, pr and her2 Receptor Status Between Primary and Metastatic Tumor

	ER			PR			HER2		
Status	Change (Overall)	Pos-neg (Loss)	Neg-pos (Gain)	Change (Overall)	Pos-neg (Loss)	Neg-pos (Gain)	Change (Overall)	Pos-neg (Loss)	Neg-pos (Gain)
No. (%)	20/65 (30.7%)	4/15 (26.7%)	16/50 (32%)	28/65 (43%)	12/20 (60%)	16/45 (35.5%)	13/65 (20%)	8/25 (32%)	5/40 (12.5%)

CONCLUSION

Our study demonstrates important differences in metastatic behaviour between the breast cancer subtypes as defined by a panel of immunohistochemical markers and contributes to an expanding knowledge of prognostic and predictive markers that will allow individualized therapy for metastatic breast cancer similar to current approaches in development for early-stage disease.

Conflicts of Interest : None.
Source of Funding : Nil.

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