

## **Comparison of Estrogen receptors, Progesterone receptors and HER-2/neu Expression between Primary and Metastatic Breast Carcinoma**

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### **Abstract**

**Objective:** To determine whether receptor status and HER-2/neu status remains same in primary and metastatic breast carcinoma to corresponding lymph nodes of individual patients.

**Methods:** Estrogen receptors, progesterone receptors and HER-2/neu immunohistochemical stains were performed on primary and metastatic breast carcinoma to axillary lymph nodes in 100 patients. Data was collected on a Performa and age, tumour size, type, grade and expression of ER, PR and HER-2/neu on primary and metastatic tumours were recorded.

**Results:** Hormone receptor status was compared between primary and metastatic tumours. Estrogen receptor positivity was observed in 28% primary tumours which were reduced to 25% in metastatic carcinoma. Progesterone receptors were positive in 28% primary tumours as compared to 22% in metastatic tumours. Her-2/neu protein over expression was noted in 44% primary and 45% metastatic breast tumours respectively. In case to case comparison ER, PR and HER-2/neu showed 91%, 88% and 95% concordance in primary and metastatic tumours respectively.

**Conclusion:** ER, PR and HER-2/neu biomarkers showed significant concordance between primary and metastatic breast carcinoma (JPMA 59:736; 2009).

### **Introduction**

Breast cancer is a major medical problem with significant public health and societal ramifications and is a leading cause of Cancer death in women. Breast carcinoma is the most common malignant neoplasm, with more than a million cases occurring worldwide annually.<sup>1</sup>

Several risk factors for the development of breast cancer have been established, and been proposed that the common denominator for most of these factors is prolonged estrogen stimulation operating on a genetically susceptible background.<sup>2</sup> Numerous studies suggest a strong link between the female hormone, estrogen, and the development of breast cancer. There is two to three times increased risk of breast carcinoma if a first degree relative has breast cancer.<sup>3</sup> Early menarche, nulliparity, late age at first birth and late menopause are all correlated with increased risk.<sup>4</sup> According to some old studies, exogenous hormone intake increases overall risk to 2.5 fold.<sup>5</sup> More recently, a large cohort study and a case control study have provided strong evidence for a greater increase in breast cancer risk in women using hormone replacement therapy than in those using estrogen alone.<sup>6</sup> In December 2002, the hormone estrogen was declared a known human carcinogen by the National Toxicology Program. Presence of hormone receptors in tumour tissue correlates well with response to hormone therapy and chemotherapy.<sup>7</sup>

Determination of estrogen and progesterone receptors

status is helpful in selecting the patients most likely to receive benefit from endocrine therapy, and provide prognostic information on recurrence and survival since their expression is related to the degree of the tumour differentiation. The highest response rates to endocrine therapy are observed in tumours, which are positive for estrogen and progesterone receptors.<sup>8</sup> Those patients with receptor positive tumours who do suffer a recurrence usually do not have aggressive liver or central nervous system involvement and they more commonly experience a relapse in bone and soft tissue.<sup>9</sup>

The HER-2/neu proto-oncogene is amplified and as a result over expressed in 25% to 30% of human breast cancer and is usually associated with tumour aggressiveness and poor prognosis.<sup>10</sup> In breast cancer, several studies identified the value of analyzing HER-2/neu as an approach to predict the response of individual tumours to chemotherapy as well as in the use of recombinant humanized antibodies (trastuzumab) to the HER-2/neu protein in the active management of patients with metastatic breast disease.<sup>11</sup>

Studies show significant concordance of ER and PR expression between primary and metastatic breast carcinoma to axillary lymph nodes.<sup>12-15</sup> However there is slight decrease in expression of these biomarkers 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 lose estrogen and progesterone receptors during the process of metastasis. HER-2/neu expression, however, remains almost same in primary and metastatic breast carcinomas.<sup>16,17</sup> The presented study compares the expression of these biomarkers between primary and metastatic breast carcinoma.

## Patients and Methods

One hundred patients of breast carcinoma with lymph node metastases were selected for study from June 2004 to September 2006 at pathology department Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan. Mastectomy specimens of patients having primary tumours and involved lymph nodes were included in the study. Poorly preserved specimens were excluded. ER, PR and HER-2/neu immunohistochemical stains were performed on primary and metastatic breast tumours. Patients' information was collected on a Performa.

Mastectomy specimens were sliced and fixed in 10% buffered neutral formalin. Size of the tumour was recorded and representative sections were taken from primary tumours. Lymph nodes were dissected out from axillary tissue and submitted. Sections were processed, cut and stained with haematoxylin and eosin. Appropriate sections from primary and metastatic tumours were selected for ER, PR and HER-2/neu immunohistochemical stains. Thin sections (4 µm) were cut. Immunohistochemical stains were performed according to manufacturer's specifications.

*Reporting criteria:* A "quick score" method<sup>18</sup> was used for the quantification of Estrogen and progesterone receptors. The quick score categories were based on both the intensity and the proportion of brown nuclear staining. Two categories A and B were made. In category "A" the proportion of positive cells was assessed and scores were assigned from 1-6 according to percentage of positive cells (1=0-4%; 2=5-19%; 3=20-39%; 4=40-59%; 5=60-79%; 6=80-100%).

The whole section was scanned at low power in order to assess the general level of intensity throughout. The average intensity corresponding to the presence of negative, weak, intermediate and strong staining, was given a score from 0-3 respectively, and termed as category B. Category "A" was multiplied by category "B". A score of 0 to 18 was obtained. The score equal or more than 3 was considered as positive.

HER-2/neu was considered positive if more than 10% cells showed moderate or strong complete membranous staining. Weak, incomplete membrane staining or cytoplasmic staining was considered negative.

*Data Collection:* ER, PR and HER-2/neu immunohistochemical stains were performed on primary and metastatic breast tumours. Information was collected on a Performa. Patient's name, age, histology numbers, size of

primary tumour, type of primary tumour, grade of tumour, number of involved lymph nodes, size of largest metastatic deposit and expression of ER, PR and HER-2/neu on primary and metastatic tumour were recorded. Later on a master data sheet was developed and all the information was entered.

*Statistical Analysis:* The comparison of the distribution of histopathological characteristics between primary breast and metastatic breast tumours was made using Fisher's exact test for all biomarkers; estrogen receptors, progesterone receptors and HER-2/neu. The exact Wilcoxon rank-sum test was performed for quantitative variables. All statistical tests were two sided. All results were judged as statistically significant at a p value of 0.05 or less. All statistical computations were done using StatXact 4 for Windows (Cytel Inc., Cambridge, USA) and R, version 2.1 (R Development Core Team 2005).<sup>19</sup>

## Results

Immunohistochemical stains were performed on hundred cases. Ages of the patients ranged from 23 to 84 years with the median of 45 years. (Table-1) Mean age was  $46.27 \pm 12.18$  years. Most of the patients (73%) had ages between 30 and 59 years. Eight patients were below 29 years of age and one patient above 80 years. The tumour sizes ranged from 1.0 to 12.5 cm with mean of  $4.68 \pm 2.27$  cm. Majority of the patients (85%) had invasive ductal carcinomas, 9% had mixed tumours, 3% had invasive lobular carcinoma and 3% had mucinous carcinomas. Two to 25 lymph nodes were isolated from modified radical mastectomy

**Table-1: Pathologic Characteristics.**

Variables	Patient values
Total no of patients	100
Age of patients	
Range	23 to 84 years
Mean	$46.27 \pm 12.18$ years
Tumour Size	
Range	1.0 to 12.5 cm
Mean	$4.68 \pm 2.27$ cm
Tumour Type	
Invasive ductal carcinoma (NOS)	85 (85%)
Mixed invasive ductal carcinoma and lobular carcinoma	9 (9%)
Invasive lobular carcinoma	3 (3%)
Mucinous carcinoma	3 (3%)
Lymph node status	
No of lymph nodes isolated in each case	
Range	2.0 to 25
Mean	$13.08 \pm 5.49$
No of involved lymph nodes	
Range	1.0 to 25
Mean	$6.78 \pm 5.04$
Size of metastatic deposit	
Range	0.5 to 2.3cm
Mean	$1.3 \pm 0.51$ cm

**Table-2: Comparison of biomarkers between primary & metastatic tumours.**

Biomarkers	Primary Breast Tumor	Metastatic Tumor	P <sup>a</sup> value
	No (%)	No (%)	
Total no. of Patients	100	100	
Estrogen Receptors (ER)			
Positive	28 (28%)	25 (25%)	0.7488
Negative	72 (72%)	75 (75%)	
Progesterone Receptors (PR)			
Positive	28 (28%)	22 (22%)	0.4144
Negative	72 (72%)	78 (78%)	
HER-2/neu			
Positive	44 (44%)	45 (45%)	1.0000
Negative	56 (56%)	55 (55%)	

<sup>a</sup> Fisher's Exact Test applied

specimens with mean of  $13 \pm 5.49$  lymph nodes. The lymph nodes involved by metastatic carcinoma ranged from 1 to 25 with the mean of  $6 \pm 5.04$  lymph nodes. The size of metastatic deposits ranged from 0.5cm to 2.3cm with Mean of  $1.3 \pm 0.51$  cm (Table-1).

Hormonal receptor status was compared between primary and metastatic tumours. Estrogen receptor positivity was observed in 28% primary breast carcinomas. ER expression reduced to 25% in metastatic breast carcinoma with P value of 0.7488. Progesterone receptors were positive in 28% primary tumours with reduced expression to 22% in metastatic carcinomas. P value remained 0.4144. Her-2/neu protein over expression was noted in 44% primary breast tumours which was almost same as in the metastatic tumours. It was over expressed in 45% metastatic tumours. P value was 1.0000 (Table-2). These P values confirm that there was no statistically significant difference of biomarker expression between primary and metastatic tumours.

In case to case comparison estrogen receptors were positive both in primary and metastatic tumours in 22% cases and negative in 69% cases. Therefore 91% cases had same expression of estrogen receptors in primary and metastatic breast carcinoma. Progesterone receptors showed 19% positivity both in primary and metastatic tumours while 69% cases were negative in both with 88% agreement between primary and metastatic tumours. HER-2/neu was positive both in primary and metastatic tumours in 42% cases and negative in 53% cases with 95% concordance (Table-3).

When these stains were assessed under microscope, the hormone receptors staining ranged from very strong with quick score of 15, to weak nuclear staining patterns with quick score of 3. Few tumours show variable expression of hormonal receptors with strong, weak and negative staining within the same tumour. When comparison between primary and metastatic tumours was done, intensity and proportion of

**Table-3: Case to case comparison of biomarkers between primary & metastatic carcinoma.**

Biomarkers	Estrogen Receptors (ER)	Progesterone Receptors (PR)	HER-2/neu
	No (%)	No (%)	No (%)
Positive in both primary and metastatic carcinoma	22 (22%)	19 (19%)	42 (42%)
Negative in both primary and metastatic carcinoma	69 (69%)	69 (69%)	53 (53%)
Expression remained unchanged (Agreement/Concordance)	22+69=91 (91%)	19+69=88 (88%)	42+53=95 (95%)
Positive in primary but negative in metastatic carcinoma	6 (6%)	9 (9%)	2 (2%)
Negative in primary but positive in metastatic carcinoma	3 (3%)	3 (3%)	3 (3%)
Total discrepant cases (Discordance)	6+3=9 (9%)	9+3=12 (12%)	2+3=5 (5%)

Total number of patients = 100.

positive cells was almost same in most of the cases. HER-2/neu expression ranged from negative (1+) to weakly positive (2+) to strongly positive (3+). Metastases showed almost same proportion and intensity of HER-2/neu over expression as in primary tumours.

## Discussion

Breast cancer is a typically sex hormone dependant neoplasm and estrogen is believed to play an important role in the pathogenesis and initial proliferation of this tumour. Estrogen binds to the estrogen receptor (ER) resulting in an activated complex that acts as a transcription factor through binding to the target genes. Estrogen dependant growth can be blocked by anti-estrogen, which competes for binding to the estrogen receptor. The evaluation of ER expression in the breast tissue has brought about prominent developments in the treatment of breast cancer, especially in the area of endocrine therapy. Further more ER expression is a prognostic indicator and ER positive breast cancer patients show significantly longer survival and disease-free intervals. ER status of a patient with breast carcinoma is usually determined only on the basis of ER content in the primary carcinomas as it is generally assumed that ER content of the metastases is similar to that of primary carcinomas. If metastatic tumours have an ER status different from that of primary tumour, the implication of ER expression may differ between primary and metastatic lesions and hormone status has to be separately established in metastatic deposits. Metastatic tumours have been reported as being less frequently receptor positive than primary tumours, although there appears to be reasonable agreement in the receptor status between primary tumour and involved regional lymph nodes.

In this study, hormonal receptor status was compared between primary and metastatic tumours to the axillary lymph nodes. Estrogen receptor positivity was observed in 28% primary breast carcinomas and its expression reduced to 25% in metastatic carcinoma to the corresponding lymph nodes with P value of 0.7488. Progesterone receptors were positive in 28% primary tumours with reduced expression to 22% in metastatic carcinomas with P value of 0.4144. Both of these P values are not significant confirming that the difference of estrogen and progesterone receptor expression between primary and metastatic carcinoma is not statistically significant. When case to case comparison was done, estrogen and progesterone receptors showed 91% and 88% concordance respectively, again confirming that primary and metastatic tumours express hormonal receptors with reasonable agreement. The mild difference which is noted in this study is statistically not significant.

A number of studies compared estrogen and progesterone receptor status between primary and metastatic carcinomas to corresponding lymph nodes. In 2003, Iguchi and colleagues evaluated 87 patients for estrogen receptor expression in primary and metastatic carcinoma to the corresponding lymph nodes. Their study showed 75.9% concordance between primary tumour and metastases, 23% cases were ER positive and 52.9% were negative both in primary and metastatic tumours. ER expression differed in 24.1% cases.<sup>12</sup> In 1995, Nedergaard L evaluated 101 patients. ER status in primary and metastatic tumours was concordant in 79% (80) patients and discordant in 21% (21) cases.<sup>13</sup> In 1983 Holdaway showed 46% concordance between primary and secondary tumours for estrogen and progesterone receptors.<sup>20</sup> Mori and Morimoto in 1991 showed decreased expression and intensity of estrogen receptors in metastases to the lymph nodes.<sup>21</sup> In 1995 Van Agthoven studied 26 cases with lymph node metastases. ER was positive in 19 (73%) primary tumours and 18 (69%) lymph node metastases. PR was detected in 63% of primary tumours and 62% of lymph node metastases.<sup>15</sup> Klinga K and colleagues also showed the same results. They found 68% concordance for estrogen receptors and 74% for progesterone receptors.<sup>22</sup> Cianga C showed that 30% breast carcinomas lost their expression of hormone receptors from primary tumours to axillary metastases.<sup>23</sup> Hoehn and Plotka in 1979 got 81% agreement between primary and metastatic carcinoma for estrogen receptors.<sup>24</sup> In our study, we obtained comparable results with those described in previous studies.

ER status is one of the standard criteria for determining the indication of endocrine therapy. However, the presence of ER is no warrant for response to endocrine therapy because 40 to 50% patients with ER positive primary tumours do not respond and almost all patients ultimately develop metastases refractory to treatment. About 10% ER negative carcinomas

are hormone dependent. Many mechanisms may potentially be responsible for these controversial observations. Previous reports show that there are functional variants of ER and ER  $\beta$  which may contribute. Moreover ER status in the primary tumour and involved corresponding lymph nodes has been found to be discordant in about 15% and 30% cases. This discordance of ER status may be due to loss of estrogen receptors during the process of metastases as we see in all these studies. Tumour heterogeneity probably also plays an important role. Estrogen and progesterone receptor positive primary tumours contain small percentage of cells which do not express hormone receptors. These cells represent the poorly differentiated population of cells which have the potential to metastasize to distant sites and usually do not respond to the endocrine therapy. This view is supported by a study in which ER content of 60 primary breast carcinomas and their lymph node metastases was investigated. Samples of primary tumour were examined for ER and were classified as "positive" if all samples were positive, ER "negative" if all were negative and "heterogeneous" if some were positive and some were negative. The study showed that 17 out of 20 heterogeneous tumours (85%) had ER negative lymph node metastases. All uniformly negative tumours had negative metastases.<sup>25</sup> There is loss of Estrogen and Progesterone receptor expression in our study as well (Table-2). This observation may explain why some hormone receptor positive tumours did not respond to endocrine therapy.

HER-2/neu proto-oncogene is over expressed in 25% to 30% of human breast cancers and is usually associated with tumour aggressiveness and poor prognosis. HER-2/neu is analyzed as an approach to predict the response of individual tumours to chemotherapy as well as in the use of recombinant humanized antibodies (trastuzumab) to the HER-2/neu protein in the active management of patients with metastatic breast disease.<sup>11</sup> The main targets of any therapy in metastatic breast cancer are the metastases. However, in great majority of cases, HER-2/neu status is determined on the primary tumour. Studies have reported a high level of consistency, although not complete, in HER-2/neu between primary and loco-regional metastases using immunohistochemistry.

Her-2/neu protein over expression was noted in 44% primary breast tumours which was almost same as in the metastatic tumours. It was over expressed in 45% metastatic tumours, with no statistical difference between primary and metastatic tumours. In 1999 Tuziak and Olszewski evaluated 71 cases of breast cancer. They found HER-2/neu over expression in 40.8% primary and 40.9% metastatic tumours.<sup>16</sup> A study by Tanner and Jarvinen showed amplification in 28% of primary tumours always associated with amplification in their metastases.<sup>17</sup> In 2000, Shimizu et al evaluated HER-2 protein levels by immunohistochemistry, in primary and metastatic cancer samples from 21 patients. The authors found

no significant differences in the HER-2/neu expression between primary tumours and their metastases.<sup>26</sup> In 2000, Masood et al evaluated HER-2 over expression in 56 patients by immunohistochemistry. The pattern and intensity of Her-2 over expression were found to be almost identical, with heterogeneity present in only one case.<sup>27</sup> Our study showed 95% agreement between primary and metastases which are comparable with previous studies.

### Conclusion

Estrogen and progesterone receptors and HER-2/neu protein expression showed significant concordance between primary and metastatic breast carcinoma to the corresponding lymph nodes. Hormone receptor status is usually determined only on the basis of receptor content in the primary tumours as it is generally assumed that hormone receptor content of the metastases is similar to that of primary carcinomas. Our study favors previous concept because there is no statistically significant difference of hormone receptor expression between primary and metastatic tumours. On the other hand metastatic deposits in the lymph nodes can also be used to assess hormone receptor status in the third world setting, when tissue from the primary tumour is not available for receptor analysis. However there are a small percentage of ER and PR positive patients in which metastatic deposits do not express hormone receptors. Therefore the patients who developed distant metastasis despite the endocrine therapy may undergo radiologically assisted biopsies and get hormone assays done in order to know the exact status of the hormone receptor expression.

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