Original Article

A study of correlation between molecular subtypes of breast cancer and site of metastasis

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ABSTRACT

Context: Studies have correlated the presence or absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2/neu) with metastatic spread and ultimate clinical outcomes in breast cancer. However, the influence of molecular subtype on the pattern of disease spread is not well known. **Aims:** The aim of this study is to evaluate the role of various molecular subtypes as a predictor of metastatic distribution in breast carcinoma. **Settings and Design:** This was a prospective observational study. **Subjects and Methods:** One-hundred and ten patients of infiltrating ductal carcinoma of breast with distant metastasis were included in the study. Evaluation for metastasis was done using radiodiagnostics. Pathological data were obtained from previous mastectomy specimens. Tumor marker status (ER, PR, and Her2/neu) was assessed, and patients were classified into Luminal A, Luminal B, Her2 enriched, and triple negative. Chi-square test was used to check the relationship between metastasis and different molecular subtypes ($P < 0.05$ was considered statistically significant). **Statistical Analysis Used:** Chi-square test was performed. **Results:** About 44.6% cases were Luminal A, followed by Luminal B (26.4%), triple negative (18.2%), and Her2 enriched (10.9%). Metastasis was seen in bones (62.7%), lungs (38.2%), liver (27.3%), and brain (10.9%). Luminal A breast cancers metastasized most commonly to bones (71.4%), lungs (36.7%), liver (18.4%), and brain (8.2%) ($P = 0.0001$). Luminal B type spread to bones in 62.1% cases, followed by liver (37.9%), lungs (34.5%), and brain (10.3%) ($P = 0.001$). Triple negative type cancers involved bones (60%), lungs (50%), liver (20%), and brain (10%) ($P = 0.002$). Her2-enriched cancers spread to liver (50%), followed by bones and lungs (33.3% each) and brain (25%) ($P = 0.630$). **Conclusions:** The major molecular subtypes in breast cancer are evidently different with regard to their ability to metastasize to different organs.

Key words: Breast cancer, metastasis, molecular subtypes

INTRODUCTION

Breast cancers are characterized by high production of growth factors and hormone receptors.[1] Receptor status was traditionally considered by reviewing each receptor (estrogen receptor [ER], progesterone receptor [PR], and human epidermal growth factor receptor 2 [Her2]) separately. Newer approaches look at these together, to categorize breast cancer into several conceptual subgroups that have different behavior, prognoses, and may have different responses to specific therapies. In brief, breast cancers are classified into subtypes through immunohistochemistry as Luminal A, Luminal B, Her2-enriched, and triple-negative breast cancer.[2]

Despite the progress that has been made in the diagnosis and treatment of early-stage breast cancer, a substantial

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Access this article online

Quick Response Code:

Website: www.ccij-online.org

DOI: 10.4103/2278-0513.197864

Cite this article as: Chauhan A, Sachan PK, Chauhan N. A study of correlation between molecular subtypes of breast cancer and site of metastasis. Clin Cancer Investig J 2016;5:393-7.

proportion of patients still go on to develop incurable distant metastatic disease.[3] The heterogeneous nature of breast cancer makes it difficult not only to define cure for this disease but also to assess risk factors for metastasis.[4]

Metastasis of breast cancer is influenced by stage at initial presentation as well as the intrinsic biology of the tumor (e.g., grade, lymphovascular invasion, ER, and Her2 status).[5-8] A number of studies have correlated the presence or absence of ER, PR, and Her2/neu with metastatic spread and ultimate clinical outcomes.[8,9] However, the influence of molecular subtype on the pattern of disease spread is not well known.

The study was undertaken to evaluate the role of various molecular subtypes as a predictor of metastatic distribution in breast carcinoma.

SUBJECTS AND METHODS

A prospective, observational study was carried out in a Tertiary Care Hospital in Uttarakhand, India, over period of 1 year in 2014. Ethical clearance was obtained from the Institutional Ethics Committee. One-hundred and ten patients suffering from metastatic disease following previous diagnosis of infiltrating ductal carcinoma of breast and patients with metastatic disease at the time of initial diagnosis of breast cancer were enrolled in the study. Patients not giving consent for investigations and workup and having any pathology other than infiltrating ductal type were excluded from the study.

Data were collected from patients and previous hospital records, investigation reports, e.g., mammogram and histopathology. Evaluation for metastasis was done in all cases. It included chest X-ray for lungs and pleural metastasis, ultrasound of abdomen for liver metastasis, and bone scan for bony metastasis. Computed tomography thorax, brain, or abdomen) was done if clinically indicated or results were uncertain or equivocal. Any new metastasis found in previously recruited patients on follow-up was noted.

Pathological data were obtained from the patients' previous mastectomy or wide local excision specimens. Histological grade, lymph node status, tumor size, multicentricity, perineural, and lymphovascular invasion were recorded for each patient.

Tumor marker status (ER, PR, and Her2/neu) was assessed in all the cases. Immunohistochemistry was done using BioGenex-anti-ER (1D5) for ER status, BioGenex-anti-PR88 for PR status, and BioGenex anti-Erb B-2/Her2 (EP1045Y) for Her2/neu status assessment.

Patients were classified into four molecular subtypes as follows: Luminal A (ER+, PR+, and Her2−), Luminal B (ER+, PR+, and Her2+), Her2 enriched (ER−, PR−, and Her2+), and basal-like/triple negative (ER−, PR−, and Her2−).

SPSS Statistics V 22.0 (IBM, New York, USA) was used for statistical analysis. Qualitative data (mammogram reports, histopathology reports, etc.) were represented in the form of frequency and percentage. Quantitative data (age, size of tumor, etc.) were represented in the form of mean ± standard deviation. Chi-square test was used to check the test of independence between metastasis and different molecular subtypes. Statistical significance was checked at 5% level of significance or $P < 0.05$ was considered statistically significant.

RESULTS

All the patients were female and had infiltrating ductal carcinoma of breast. About 63.6% of the patients belonged to age group between 45 and 64 years and the mean age of patients was 50.5 years.

The "T" stage of tumor at time of diagnosis of malignancy was T_4 in 40.9%, T_3 in 19.0%, T_2 in 37.3%, and T_1 in 2.7%. Clinically, 23.6% of patients had no lymph node metastasis. Nearly 70% of the patients had palpable ipsilateral axillary lymphadenopathy (mobile/fixed).

Almost 68.2% of the metastatic infiltrating ductal carcinoma cases were of Grade II. About 28.2% were Grade III and only 3.6% were Grade I.

Of 110 cases, 70.9% cases were ER+ and 29.1% cases were ER−. About 60.9% of the cases showed positivity toward PRs and 39.1% were PR−. Her2/neu positivity was observed in 37.3% cases and 62.7% were Her2/neu−. Detailed characteristics of patients and tumor are shown in Tables 1 and 2.

Among the four molecular subtypes of breast cancer, 49 (44.6%) were Luminal A type and 29 (26.4%) were Luminal B. A total of 20 (18.2%) cases were triple negative and 12 (10.9%) were Her2 enriched.

Metastasis to bones and lungs was seen in 69 (62.7%) and 42 (38.2%) patients, respectively. Metastasis to liver was observed in 30 (27.3%) and brain was involved in 12 (10.9%) patients. Involvement of adrenal was found in 2 (1.8%) cases, ovaries in 1 (0.9%) case, and peritoneum in 1 (0.9%) case.

Table 3 demonstrates correlation between the four molecular subtypes of breast cancer and site of metastasis in our patients. Luminal A breast cancers were found

to metastasize most commonly to bones (71.4%). The second most common site was lungs (36.7%) followed by liver (18.4%) and then brain (8.2%). This proportion was statistically significant (P = 0.0001) at 5% level of significance.

Luminal B type spread to bones in 62.1% cases followed by liver (37.9%). Metastasis to lungs was seen in 34.5% and to brain in 10.3%. This difference was statistically significant (P = 0.001).

Triple-negative breast cancers spread to bones in 60% and to lungs in 50% cases. Metastasis to liver was observed in 20% and to brain was seen in 10%. At 5% level of significance, this observed proportion was found to be statistically significant (P = 0.002).

Breast cancers that were Her2 enriched most commonly spread to liver (50%). Spread to bones and lungs was observed in 33.3% each, and to brain was found in 25% patients. However, the observed difference was not statistically significant (P = 0.630).

Table 1: Clinical characteristics of the patients and tumor (*n*=110)

Parameters	*n* (%)
Age (years)	
25-44	28 (25.4)
45-64	70 (63.6)
65-84	12 (10.9)
Side	
Left	58 (52.7)
Right	52 (47.3)
Stage	
T1	3 (2.7)
T2	41 (37.3)
T3	21 (19.0)
T4	45 (40.9)
N0	26 (23.6)
N1	42 (38.2)
N2	35 (31.8)
N3	7 (6.4)

Table 2: Pathological characteristics of the tumor (*n*=110)

Parameters	*n* (%)
Grade	
I	4 (3.6)
II	75 (68.2)
III	31 (28.2)
Multicentricity	
Present	6 (5.4)
Absent	87 (79.1)
Unknown	17 (15.5)
Lymphovascular invasion	
Present	20 (18.2)
Absent	62 (56.4)
Unknown	28 (25.4)
Perineural invasion	
Present	20 (18.2)
Absent	62 (56.4)
Unknown	28 (25.4)
Estrogen receptor	
Positive	78 (70.9)
Negative	32 (29.1)
Progesterone receptor	
Positive	67 (60.9)
Negative	43 (39.1)
Her2/neu	
Positive	41 (37.3)
Negative	69 (62.7)

DISCUSSION

Perou *et al.*[10] described the presence of various molecular subtypes of breast cancer that had a difference in expression of hormonal receptors and molecular markers. They were also different in their aggressiveness and response to specific chemotherapy. In our study, we aimed to determine relation between molecular subtypes and metastatic spread to specific distant organs.

A large number of patients (63.6%) belonged to age group between 45 and 64 years. The mean age of patients in the study was 50.5 years. This is similar to Murthy *et al.*[11] who reported the mean age of breast cancer patients in India as 50 years. About 5.5% patients in our study had age <34 years.

The majority of the cases in our study had a high "T" stage (T_{3-4}) (59.9%) and "N" stage. Most of the studies have reported lower "T" and "N" stage of the tumor at diagnosis.[9,12,13] This discrepancy in findings may be attributed to late presentation of cases to hospital in our study and lack of awareness about breast cancer among

Table 3: Correlation between molecular subtypes and site of metastasis

Molecular subtypes (*n*=110)	Involvement	Organ involved				χ^2	*P*
		Bones (%)	Lungs (%)	Liver (%)	Brain (%)		
Luminal A (*n*=49)	Yes	35 (71.4)	18 (36.7)	9 (18.4)	4 (8.2)	50.9	0.0001
	No	14 (28.6)	31 (63.3)	40 (81.6)	45 (91.8)		
Luminal B (*n*=29)	Yes	18 (62.1)	10 (34.5)	11 (37.9)	3 (10.3)	16.1	0.001
	No	11 (37.9)	19 (65.5)	18 (62.1)	26 (89.7)		
Triple negative (*n*=20)	Yes	12 (60.0)	10 (50.0)	4 (20.0)	2 (10.0)	14.9	0.002
	No	8 (40.0)	10 (50.0)	16 (80.0)	18 (90.0)		
Her2 enriched (*n*=12)	Yes	4 (33.3)	4 (33.3)	6 (50.0)	3 (25.0)	1.73	0.630
	No	8 (66.7)	8 (66.7)	6 (50.0)	9 (75.0)		

suburban and rural Indian population to which majority of cases belonged.

Most of the breast cancers were of Grade II and III (96.4%) in our study. Porter *et al*.[14] had 92.5% of Grade II and III breast cancer in their study.

Breast cancer ER positivity has been reported from 72% to 86%, PR positivity as 59%–75%, and Her2/neu positivity as 17%–23% in various studies.[12,15,16] In our research, incidence of estrogen and PR positivity was similar to other studies, but incidence of Her2/neu positivity was slightly higher.

Among the four molecular subtypes of breast cancer, majority of the metastatic cases in our study were Luminal A (44.6%). The second most common type was Luminal B (26.4%). This was followed by triple-negative (18.2%) and Her2-enriched cases (10.9%). Verma *et al*.[17] reported incidence of Luminal A as 47%, Luminal B 15%, Her2 enriched 21%, and triple negative 17%. Onitilo *et al*.[18] had reported 7.5% cases as Her2 enriched and 13.4% of the cases as basal type (triple negative).

Bones were the most common site of distant metastasis, followed by lungs, liver, and brain in decreasing order of incidence. Many authors have reported similar observations.[12,16,18,19]

Luminal A breast cancers were found to metastasize most commonly to bones. The second most common site was lungs followed by liver and brain. Smid *et al*.[20] found that Luminal A had metastasized to bones in 64.7% cases, lungs and pleura in 20.5%, liver in 11.8%, and to brain in 2.9%. Similarly Kennecke *et al*.[13] reported incidence of bone metastasis in Luminal A to be 66.6%, lungs in 23.8%, liver in 28.6%, and brain in 7.6%.

Metastatic pattern of Luminal B tumors in our study population was found to be similar to other studies. Smid *et al*.[20] reported it to be 57.8% in bones, 33.3% in lungs and pleura, 4.4% in liver, and 2.2% in brain. In the study by Kennecke *et al*.,[13] bones were involved in 71.4%, lungs in 30.4%, liver in 32%, and brain in 10.8%.

In study by Smid *et al*.,[20] metastasis of basal type (triple negative) breast cancers to bones was seen in 16.6%, lungs in 43.3%, liver in 13.3%, and brain in 26.6%. In Kennecke *et al*.'s study,[13] basal type (triple negative) most commonly metastasized to lungs (42.8%) then bones (39%) followed by brain (25.2%) and liver (21.4%). Triple-negative breast cancers in our study had increased tendency to spread to bones and lungs, and less tendency of spreading to liver. However, our results differed in relation to brain metastasis. We had 10% metastasis to brain in triple-negative breast cancers.

According to Smid *et al*.,[20] bone involvement was seen in 51.9%, lungs 14.8%, liver 22.2%, and brain 11.1% in Her2-enriched breast cancers. Kennecke *et al*.[13] found bony metastasis in 59.6% and lung metastasis in 47.1%. Liver was involved in 45.6% and brain in 28.7% cases. Increased tendency of Her2/neu-overexpressing tumors to metastasize to brain was also shown by Gaedcke *et al*.[21] Although our data were not statistically significant, increased predisposition of Her2/neu-enriched breast cancers to spread to liver and brain could be appreciated.

Smid *et al*.[20] studied genetic expressions in these molecular subtypes and concluded that site of metastasis was not randomly distributed across the subtypes. Each molecular subtype expressed different set of genes. This finding supported the view that specific biological processes are involved in organ-specific metastasis.

Thus, from our study, it can be concluded that the major molecular subtypes as defined by panel of immunohistochemical markers in breast cancer are evidently different with regard to their ability to metastasize to different organs. Appreciation of these distributions can aid the radiologist in detecting metastatic lesions and will help the clinician estimate the likelihood of metastases to various organ systems, as well as to potentially target therapy.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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