Original Research Article

A cross-sectional study on the pattern of recurrence in carcinoma breast patients based on molecular subtypes

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ABSTRACT

Background: Breast cancer is a major public health issue and it is the leading cause of cancer related death in females worldwide. New insights in the cancer treatment led to considerable improvement in the survival of cancer patients. But metastasis remain an area were all sorts of conventional treatments fails and it is the cause of death of most carcinoma breast patients. In this study we aim to establish a possible link to local recurrence and distant metastasis with different biological subtypes of breast cancer.
Methods: One hundred and eighty patients of carcinoma breast patients of carcinoma breast who presented with local recurrence or distant metastasis in the period of January 2018 to March 2019 in Government Medical College, Thiruvananthapuram were included in this study. These data were collected from the hospital records.
Results: Local recurrence was most seen in triple negative (50%) subtype followed by HER2 (32.1%) enriched. Local recurrence was least among luminal A (13.8%) with a p value of 0.001. Bone metastasis was the most common type of metastasis and was most seen in luminal A (p=0.001). Triple negative had the maximum CNS metastasis with a p value of 0.003. Liver metastasis was seen mostly in luminal B (26.2%) and A (20.7%) and lung metastasis in triple negative (13.5%) and HER2/neu (10.7%). However, there was no significant association for lung or liver metastasis to any subtype.
Conclusions: Biological subtypes of breast cancer classified by immunohistochemical expression of ER, PR, HER2, Ki 67 show different clinicopathological features, recurrence pattern, and survival outcomes.

Keywords: Carcinoma breast, Biological subtypes, Local recurrence, Metastasis, Triple negative, HER2

INTRODUCTION

Though lot of advances are made in the treatment of breast cancer, it still remains as a leading cause of cancer related death in females.[1] We can find descriptions of breast malignancies in ancient documents which date back to 5th century BC.[2] Like many other diseases it was also considered as punishment to one's sins.[3] Evolution of breast surgery happened over centuries and we witnessed a lot of changes happening in last few decades.[4]

Breast cancer is a heterogeneous disease with widely varying clinical behavior and treatment responses.[5] Discovery of various subtypes was a breakthrough in the research of carcinoma breast which led to the hypothesis that these biological subtypes may determine the course of the disease and it may give the opportunity for the treating physician an additional tool for targeted therapy.[6] At least four major subtypes of breast cancer are elucidated through gene expression profiling. These include luminal A, luminal B, HER2 enriched, and basal like. Although exact definitions of these subtypes are an area of active debate and the definitions are yet to be

defined, it is certain that these subtypes are reproducible in multiple unrelated data sets, and their prognostic impacts has been validated in these settings.[7]

In this study we evaluated organ specific metastasis and local recurrence in 180 carcinoma breast patients according to their estrogen receptor (ER), progesterone receptor and human epidermal growth factor receptor – 2 (HER2)/neu expression data.

METHODS

One hundred and eighty patients of carcinoma breast patients of carcinoma breast who presented with local recurrence or distant metastasis in the period of January 2018 to March 2019 in Government Medical College, Thiruvananthapuram were included in this study. The data were collected from the hospital records. All the investigation reports that is diagnostic were noted; for example, sonomammography, histopathological reports, ER, PR and HER2/neu status. Work up for metastasis were reviewed which included chest X-ray/CECT chest for lung metastasis, ultrasound/CECT of pelvis and abdomen for liver or other visceral metastasis, and bone scan for bony metastasis. Magnetic resonance imaging or CT scan of the brain was used for diagnosis of metastasis.

All patients within the study were either local recurrence or metastatic. Patients undergone either modified radical mastectomy (MRM) or breast conservation surgery (BCS) as the primary surgical treatment modality followed by adjuvant chemotherapy and/or radiotherapy. Patients were stratified according to pattern of recurrence in relation to biological subtypes of cancer.

We evaluated the ER, PR, and HER-2/Neu expression of the patients; first to classify them into the molecular subtypes, then to analyse which specific subtype showed what organ-specific metastasis. The ER, PR and HER-2/Neu status of all patients were noted and then the patients were classified accordingly into luminal A/B, HER2/Neu enriched and triple negative. Breast cancer molecular subtypes were classified based on a gene expression profile validated immunohistochemical (IHC) surrogate panel into the following: luminal A: ER+, and/or PR+, and HER2/neu- luminal B: ER+, and/or PR+, and HER2/neu-, Ki67 high or ER+, HER2/neu+, any Ki67. HER2 enriched: ER-, PR-, and HER2+ triple negative: ER-, PR-, and HER2- IHC staining was performed for ER, PR, HER2, by fully automated machine.

ER positivity and PR positivity were defined as any positive nuclear staining (≥1%) using Alldred scoring system, and HER2-positive cases were defined by positive membranous scoring. For HER2/neu, IHC score of 3+ or 2+ plus fluorescent in situ hybridization (FISH) with amplification ratio ≥2.0 was considered to be positive.

A local recurrence is defined as any chest wall recurrence in those who underwent mastectomy; any recurrence in the ipsilateral breast following surgery; and any regional lymph node basin recurrence- axillary, supraclavicular, or infraclavicular.

A local recurrence in more than one of these locations simultaneously was counted as a single recurrence for the purposes of data collection and reporting. Any other recurrence other than these sites are considered as distant metastasis.

Distant relapse was defined as recurrences of breast cancer beyond the confines of the ipsilateral breast, chest wall, or regional lymph nodes. Sites of distant relapse were categorized as follows: brain, liver, lung, bone (including bone marrow), others - pleural, peritoneal, skin outside of breast/chest wall, ovaries, spinal cord, eye, heart, and other organs not elsewhere classified, distant nodal (nodes beyond the ipsilateral axillary/ supraclavicular internal mammary area).

Numerical data were expressed as mean±SD (standard deviation) categorical data as frequencies and percentage. Association between categorical variables were analyzed using Chi-square test. All statistical tests were two sided. A p value <0.05 was considered as statistically significant. Data analysis was performed using SPSS (version 16.0).

RESULTS

All the patients in this study group presented to the hospital with evidence of relapse after primary treatment- either local recurrence or distant metastases. Total number of patients in this study is one hundred and eighty. The mean age was 53. The grade of the tumour as well as the ER, PR, HER2/neu was assessed from the resected specimen after histopathological examination. The major histology was invasive ductal carcinoma (90.6%). Most of the patients had either grade 3 (51.7%) or grade 2 (45%).

They were further classified into molecular subtypes according to their ER, PR and HER2/neu status. As shown in the data's shown luminal A was the most common molecular subtype (32.2%) followed by triple negative (28.9%). HER2/neu subtype had the lowest prevalence in our study (Figure 1). The recurrence pattern of the patients was assessed among the patients. Bone recurrence was the most common (42.8%) followed by local recurrence (30.6%) (Figure 2).
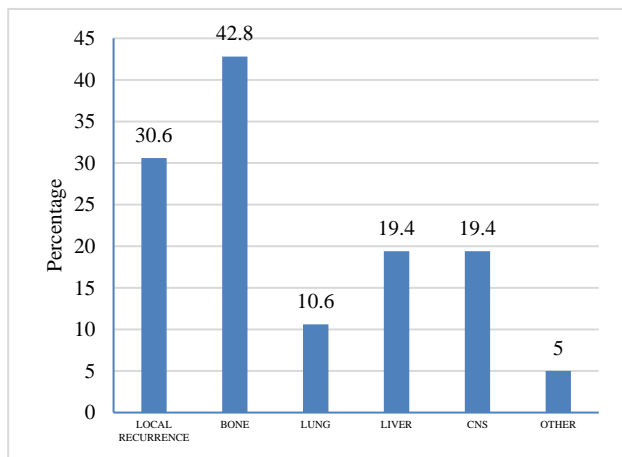
Pattern of local recurrence according to molecular subtype

Local recurrence was most seen in patients with triple negative subtype with 50% prevalence among them followed by HER2 enriched (32.1%). Local recurrence was least among luminal A (13.8%). The p value was
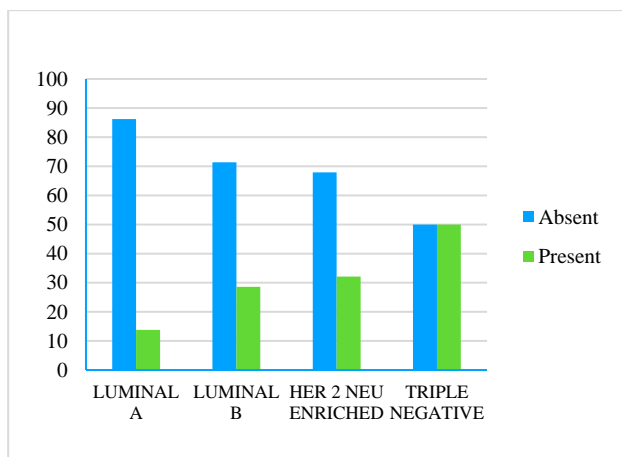
0.001 which shows a significant association between incidence of local recurrence and molecular subtypes (Table 1 and Figure 3).
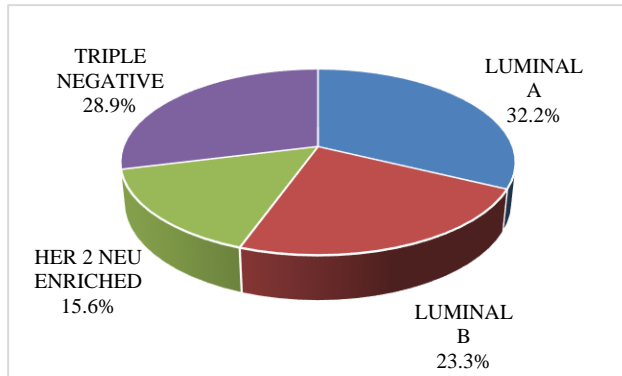
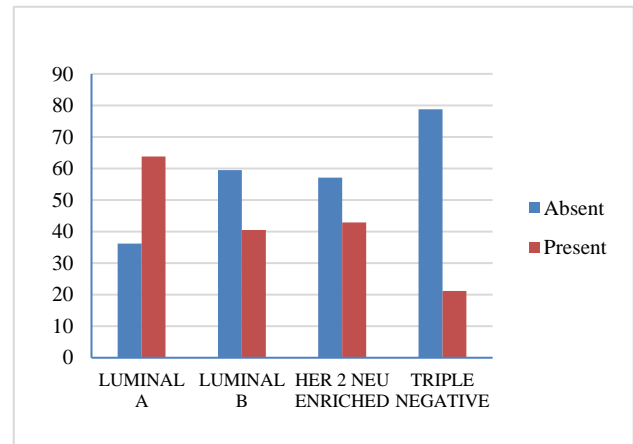**Figure 1: Molecular subtype.**

Figure 2: Recurrence.

Figure 3: Local recurrence.

Pattern of bone recurrence according to molecular subtype

Bony metastasis is the most common metastasis seen in our study. Bony metastasis was most seen in luminal A

(63.8%) followed by HER2 enriched (42.9%). The p value was <0.001 which shows a significant association between incidence of bony metastasis and molecular subtypes (Figure 4).

Figure 4: Bone metastasis.

Pattern of CNS recurrence according to molecular subtype

Triple negative had the maximum incidence of CNS metastasis. The least incidence was seen among luminal A and B. The p value was 0.003 (Figure 5).
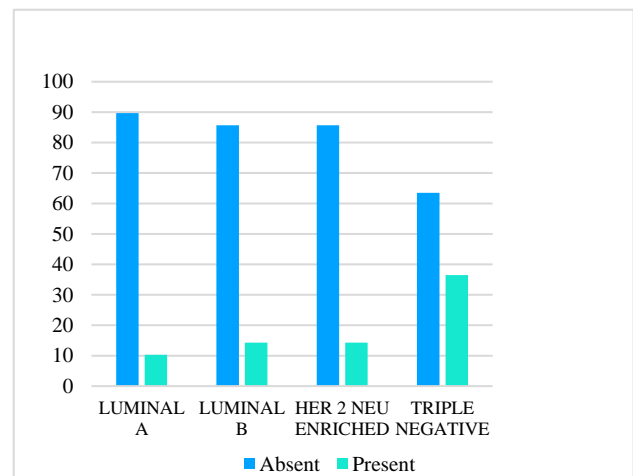
Figure 5: CNS metastasis.

Pattern of liver, lung, and other metastasis with molecular subtypes

Liver metastasis was seen mostly in luminal B (26.2%) and A (20.7%) and lung metastasis in triple negative (13.5%) and HER2/neu (10.7%). However, there was no significant association seen with any subtype. Other metastasis included contralateral side axillary node metastasis (found in 6 patients), peritoneal deposits seen in 2 patients and ovarian metastasis seen in 1 patient. These rare sites of metastasis were seen mostly in HER2/neu and triple negative patients.

Table 1: Analysis of local recurrence.

Molecular subtype	Local recurrence				Total		χ^2	df	P value
	Absent		Present						
	N	%	N	%	N	%			
Luminal A	50	86.2	8	13.8	58	100			
Luminal B	30	71.4	12	28.6	42	100			
HER2/neu enriched	19	67.9	9	32.1	28	100	17.06	3	0.001
Triple negative	26	50	26	50	52	100			
Total	125	69.4	55	30.6	180	100			

Table 2: Analysis of bone recurrence.

Molecular subtype	Bone				Total		χ^2	df	P value
	Absent		Present						
	N	%	N	%	N	%			
Luminal A	21	36.2	37	63.8	58	100			
Luminal B	25	59.5	17	40.5	42	100			
HER2/neu enriched	16	57.1	12	42.9	28	100	20.49	3	<0.001
Triple negative	41	78.8	11	21.2	52	100			
Total	103	57.2	77	42.8	180	100			

Table 3: Analysis of CNS recurrence.

Molecular subtype	CNS				Total		χ^2	df	P value
	Absent		Present						
	N	%	N	%	N	%			
Luminal A	52	89.7	6	10.3	58	100			
Luminal B	36	85.7	6	14.3	42	100			
HER2/neu enriched	24	85.7	4	14.3	28	100	13.96	3	0.003
Triple negative	33	63.5	19	36.5	52	100			
Total	145	80.6	35	19.4	180	100			

DISCUSSION

Invasion and metastasis are acquired capabilities of malignant cells which makes them what it is. Sooner or later during the course of disease tumour cells move out invade adjacent tissue then travel to distant sites where they may succeed in making new colonies.[8] These distant settlements - metastasis are the most common cause of human cancer death. Mechanisms of tumour invasion and metastasis are exceedingly complex mechanisms which still remain incompletely understood.

Biological subtypes of cancer may play a role in determining the course of disease including local recurrence and distant metastasis.[9] In 2011, endocrine responsiveness was for the first time linked to the intrinsic molecular breast cancer subtypes suggested by Perou et al.[6] Definitions of theses subtypes are still evolving. According to ESMO guidelines updates in April 2019 basal like lesions are described as triple negative.[10] There is considerable argument in the scientific community whether triple negative status alone may not be sufficient to label as basal like and how best to define these tumours is a matter of controversy and ongoing debate.[7] Immunohistochemical marker panels

that have been proposed to define basal-like breast cancers include: lack of ER, PR, and HER2 expression ('triple-negative' immunophenotype); expression of one or more high-molecular-weight/ basal cytokeratins (CK5/6, CK14, and CK17); lack of expression of ER and HER2 in conjunction with expression of CK5/6 and/or epidermal growth factor receptor (EGFR) and lack of expression of ER, PR, and HER2 in conjunction with expression of CK5/6 and/or EGFR.

One major limitation of this study is that all the triple negative cases are considered as basal like subtype. Majority of study population belonged to luminal-A subtype with least prevalent being HER2 enriched. The second common type was triple negative breast cancer (28.9%). It is to be noted that worldwide prevalence of triple negative breast cancer (TNBC) comes between 15% to 20%. In literature maximum prevalence of TNBC is described in African American women. Many studies from India also shows that prevalence of TNBC among Indian women is the highest (27.9%) compared with those from other regions of the world.[11]

Radiotherapy plays a major role in decreasing local recurrence and improves the survival in breast cancer.

Irrespective of the selection of radiotherapy paradigm, luminal A breast cancer has an overall favorable prognosis relative to HER2-positive and TNBC subtypes partially due to individual radio sensitivity of these subtypes. HER2-positive breast cancer has high radio resistance that is correlated to the transactivation of the NFκB-mediated HER2 promoter inducing HER2 overexpression, β-catenin expression during EMT (epithelial-mesenchymal transformation) and the Fak-mediated pathway.[12] In this study also local recurrence was most seen with triple negative subtype followed by HER2 enriched. Local recurrence was least among luminal A (p<0.05).

Breast cancer metastasis to bone has been extensively studied since skeleton is the most common type of metastasis.[13] Strong association between bone metastasis and hormone receptor status was observed more than two decades.[14,21] With the arrival of new data from molecular profiling now it is more evident that bone relapse is more common with luminal subtypes.[15,16] In this study also bone was the most common site of metastasis and luminal A type showed a significant association with bone metastasis with a p value <0.05.

There is an increasing rate of brain metastasis is reported in recent years especially in triple negative and HRE2 enriched.[17-19] Increase in brain metastasis be due to improved survival in triple negative and HER2 tumours with chemotherapy and trastuzumab based treatment.[20] Brain metastases are associated most frequently with high expression of nestin, prominin-1, or CK-5, but low expressed ER or PR.[13,21] It is also demonstrated that HER2 gene in metastatic sites are acquired in approximately 20% of HER2-negative primary cases.[22] In our analysis also there is a significant (p- 0.003) association between triple negative and HER2 enriched type with brain metastasis. Liver metastasis was seen mostly in luminal B (26.2%) and A (20.7%) and lung metastasis in Triple negative (13.5%) and Her2/neu (10.7%). However, there was no significant association seen with any subtype.

Despite improvement in breast cancer outcome, distant recurrence remains an area where all treatment options fail. It now clear that different biological subtypes play an important role in determining the site of distant relapse. Deeper knowledge in the molecular mechanisms will help understanding prognostic and predictive markers that will allow individualized therapy for metastatic breast cancer.

CONCLUSION

Biological subtypes of breast cancer classified by immunohistochemical expression of ER, PR, HER2, Ki 67 show different clinicopathological features, recurrence pattern, and survival outcomes.

REFERENCES

1. Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, et al. Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin. 2019;69(5):363-85.
2. Retief FP, Cilliers L. Breast cancer in antiquity. S Afr Med J. 2011;101(8):513-5.
3. Sakorafas GH, Safioleas M. Breast cancer surgery: an historical narrative. Part I. From prehistoric times to Renaissance. Eur J Cancer Care (Engl). 2009;18(6):530-44.
4. Halsted CP, Benson JR, Jatoi I. A historical account of breast cancer surgery: beware of local recurrence but be not radical. Future Oncol. 2014;10(9):1649-57.
5. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001;98(19):10869-74.
6. Perou CM, Sorlie T, Eisen MB, Rijn VDM, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000;406(6797):747-52.
7. Badve S, Dabbs DJ, Schnitt SJ, Baehner FL, Decker T, Eusebi V, et al. Basal-like and triple-negative breast cancers: a critical review with an emphasis on the implications for pathologists and oncologists. Mod Pathol. 2011;24(2):157-67.
8. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100(1):57-70.
9. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA. 2001;98(19):10869-74.
10. Cardoso F, Kyriakides S, Ohno S, Llorca PF, Poortmans P, Rubio IT, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2019;30(8):1194-220.
11. Thakur KK, Bordoloi D, Kunnumakkara AB. Alarming Burden of Triple-Negative Breast Cancer in India. Clin Breast Cancer. 2018;18(3):393-9.
12. He L, Lv Y, Song Y, Zhang B. The prognosis comparison of different molecular subtypes of breast tumors after radiotherapy and the intrinsic reasons for their distinct radiosensitivity. Cancer Manag Res. 2019;11:5765-75.
13. Wu Q, Li J, Zhu S, Wu J, Chen C, Liu Q, et al. Breast cancer subtypes predict the preferential site of distant metastases: a SEER based study. Oncotarget. 2017;8(17):27990-6.
14. Hess KR, Pusztai L, Buzdar AU, Hortobagyi GN. Estrogen receptors and distinct patterns of breast

cancer relapse. Breast Cancer Res Treat. 2003;78(1):105-18.

15. Chiu JH, Wen CS, Wang JY, Hsu CY, Tsai YF, Hung SC, et al. Role of estrogen receptors and Src signaling in mechanisms of bone metastasis by estrogen receptor positive breast cancers. J Transl Med. 2017;15(1):97.

16. Arciero CA, Guo Y, Jiang R, Behera M, Regan OR, Peng L, et al. ER+/HER2+ Breast Cancer Has Different Metastatic Patterns and Better Survival Than ER-/HER2+ Breast Cancer. Clin Breast Cancer. 2019;19(4):236-45.

17. Darlix A, Louvel G, Fraisse J, Jacot W, Brain E, Debled M, et al. Impact of breast cancer molecular subtypes on the incidence, kinetics and prognosis of central nervous system metastases in a large multicentre real-life cohort. Br J Cancer. 2019;121(12):991-1000.

18. Jones LB. Human epidermal growth factor receptor 2-positive breast cancer and central nervous system metastases. J Clin Oncol. 2009;27(31):5278-86.

19. Kunikullaya SU, Poddar J, Sharma AD, Patel S. Pattern of distant metastasis in molecular subtypes of carcinoma breast: An institutional study. Indian J Cancer. 2017;54(1):327-32.

20. Park S, Koo JS, Kim MS, Park HS, Lee JS, Lee JS, et al. Characteristics and outcomes according to molecular subtypes of breast cancer as classified by a panel of four biomarkers using immunohistochemistry. Breast. 2012;21(1):50-7.

21. Smid M, Wang Y, Zhang Y, Sieuwerts AM, Yu J, Klijn JG, et al. Subtypes of breast cancer show preferential site of relapse. Cancer Res. 2008;68(9):3108-14.

22. Priedigkeit N, Hartmaier RJ, Chen Y, Vareslija D, Basudan A, Watters RJ, et al. Intrinsic Subtype Switching and Acquired ERBB2/HER2 Amplifications and Mutations in Breast Cancer Brain Metastases. JAMA Oncol. 2017;3(5):666-71.