Research Article

Clinicopathological profile and molecular classification of breast cancer – A correlative study

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Abstract

Background: The incidence of breast cancer in India is rapidly increasing and is expected to become the most common cancer in females pushing cervical cancer to the second place. The molecular classification and treatment protocols are guided by immunostaining for estrogen receptor (ER), progesterone receptor (PR) and Her 2neu. The breast cancer cells in certain patients test negative for all these markers.These are known as triple negative cases and they do not respond to conventional therapies and have a poor prognosis. **Aims and Objectives:** To classify carcinoma breast cases based on ER,PR and Her2 neu expression pattern. To compare the clinicopathological profile of the different subtypes. **Materials and Methods:** This retrospective study was carried out on 190 invasive breast carcinoma NOS diagnosed in the Department of Pathology at Sri Ramachandra University. Histological grading was done on HandE stained sections. ER, PR and Her2 neu immunostains were performed and molecular classification was done. Clinicopathological profile of the molecular types was obtained from the case records and results analysed. **Results:** Luminal A was the most common molecular type in our series. Triple negative type presented earlier and at a higher histological grade compared to others.T2 was the most common tumor size presentation in all categories. Luminal A more commonly presented at N3 nodal status. **Conclusion:** Triple negative type presents at an earlier age and with a higher histological grade indicating a more aggressive behaviour

Key Word: Carcinoma breast, molecular classification, triple negative.

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INTRODUCTION

The incidence of breast cancer is rapidly rising worldwide, both in the developed and developing countries. The recent Indian Council of Medical Research data suggests that breast cancer will replace cervical cancer as the leading type of cancer among Indian women shortly, even in the rural areas. In 2011, over 50800 women died due to breast cancer(WHO2013). Due to the lack of early detection programmes, a high percentage of women present in late stages of the disease, resulting in a higher mortality. The major risk factors of breast cancer include early age at menarchy, nulliparity, late menopause, oral contraceptives and hormone therapy. Due to the prolonged exposure to estrogen caused by these exogenous and endogenous factors, there is an upward trend in the incidence of breast cancer. A variety of factors have been recognized to influence the prognosis of breast cancer. Advances like molecular profiling of breast cancer enable better understanding of the histology and treatment response of breast cancer. They also indicate the heterogenous nature of these cases though they are grouped as a single entity namely invasive ductal carcinoma. This observation is in line with the varying behavioural patterns of invasive ductal

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carcinoma patients. At present the treatment protocols for invasive ductal carcinoma are based on the molecular classification which in turn is based on the expression of estrogen receptor(ER), progesterone receptor(PR) and human epidermal growth factor receptor(Her2/neu).[1]

HORMONE RECEPTORS IN BREAST CANCER

Estrogen and progesterone receptors (ER and PR) have now been studied in clinical breast cancer for more than 20 years. Positive receptor status correlates with favorable prognostic features including a lower rate of cell proliferation and histologic evidence of tumor differentiation. ER and PR have their greatest utility in predicting response to hormonal therapy and adjuvant therapy for advanced disease. When the assay is done properly and cut-offs for ER-negativity and positivity are defined, receptor status is very helpful in identifying groups of patients who are likely to benefit from hormonal therapy. Tumors that express both ER and PR have the greatest benefit from hormonal therapy, but those containing only ER or only PR still have significant responses.[2] HER-2/neu is a member of the epidermal growth factor receptor (EGFR/ErbB) family. Amplification or over-expression of this gene has been shown to play an important role in the pathogenesis and progression of certain aggressive types of breast cancer3. In recent years it has evolved to become an important biomarker and also serves as a target for therapy. Amplification or overexpression occurs in approximately 30% of breast cancers. It is strongly associated with increased disease recurrence and a worse prognosis. Triple negative breast cancers are defined as tumors that lack expression of estrogen receptor (ER), progesterone receptor (PR), and HER-2/neu. Triple negative breast cancers tend to be larger than other subtypes of breast cancer and are usually high-grade.[4] Triple-negative type is more likely to metastasize to viscera, particularly to the lungs and brain, and is less likely to metastasize to bone. Multiple studies have indicated that triple negative cancers, are associated with an adverse prognosis. Women with triple-negative breast cancer do not benefit from endocrine therapy or trastuzumab.[5] Chemotherapy is currently the mainstay of systemic medical treatment. With this background we decided to study the clinicopathological profile of invasive ductal carcinoma patients and correlate it with the molecular subtypes.

AIMS AND OBJECTIVES

The aims and objectives of this study was to classify breast carcinoma cases based on ER,PR and Her2 neu expression pattern and to compare the clinicopathological profile of different molecular subtypes.

MATERIALS AND METHODS

This was a retrospective study done in the Department of Pathology, Sri Ramachandra University. An approval from the Institutional Ethics Committee was obtained prior to commencement of the study. One hundred and ninety consecutive cases of modified radical mastectomy, from 2010 to 2014 with infiltrating ductal carcinoma NOS histological subtype were included in the study. Clinical data was obtained from the medical record section.Tissue blocks were retrived from the archives of pathology department. Age of the patient, location and size of the tumor were noted. Formalin fixed paraffin embedded tumour tissue was used for the study. Five micron sections were stained with hematoxylin and eosin stain and studied. Tumors were graded using Nottingham grading system for breast cancer. Lymph node involvement was assessed and recorded. The pathological staging was done and recorded.

Following this immunohistochemical staining for ER,PR and Her2 neu was performed.
Immunostaining for ER was done using Monoclonal Antibody to Estrogen Receptor (ER), Prediluted Antibody, AbNo.272M, procured from Biogenex Laboratories.
Immunostaining for PR was done using Mouse Monoclonal Antibody to Progesterone Receptor (Clone: PR88), procured from Biogenex Laboratories.
Immunostaining for HER-2/neu was done using Monoclonal Antibody to c-erbB-2 Protein (HER-2/neu), Prediluted Antibody, AbNo.134M, procured from Biogenex Laboratories.

Tumors showing immunostain for ER and PR in the nuclei were recorded as positive. Complete membrane stain was recorded as positive for Her2neu. Based on this staining pattern molecular classification(Table.1) was done. Allred score6 was done for estrogen and progesterone receptor evaluation. HER2 results were reprted according to the College of American Pathologists (CAP) protocol7 (Table.2). Statistical analysis of the results was done by ANOVA.

RESULTS

Table 1: Molecular classification

	ER	PR	Her2neu
LUMINAL A	+	+	-
LUMINAL B	+	+	+
HER 2 NEU	-	-	+
TN	-	-	-

Table 2: Reporting of Estrogen Receptor (ER) and Progesterone Receptor (PR) Testing Result

Result	Criteria
Positive	Immunoreactive tumor cells present (≥1%)
Negative	<1% immunoreactive tumor cells present

Table 3: Reporting Results of HER2 Testing by Immunohistochemistry (IHC)

Result	Criteria
Negative (Score 0)	No staining observed *or* Incomplete, faint/barely perceptible membrane staining in ≤10% of invasive tumor cells
Negative (Score 1+)	Incomplete, faint/barely perceptible membrane staining in >10% of invasive tumor cells
Equivocal (Score 2+)	Incomplete and/or weak to moderate circumferential membrane staining in >10% of invasive tumor cells *or* Complete, intense, circumferential membrane staining in ≤10% of invasive tumor cells
Positive (Score 3+)	Complete, intense, circumferential membrane staining in >10% of invasive tumor cells

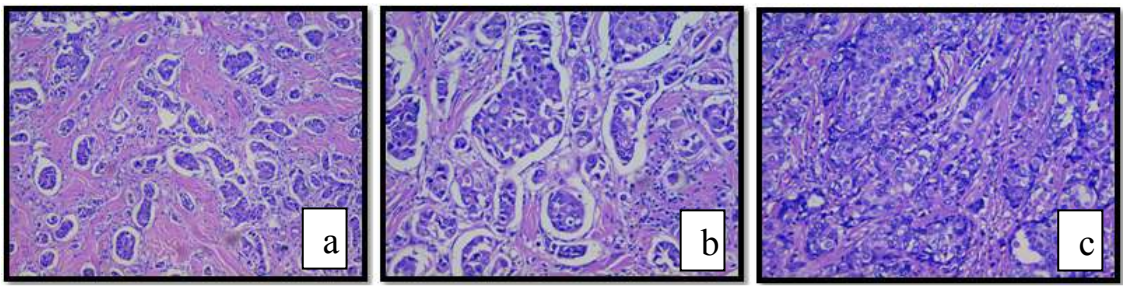


Figure 1

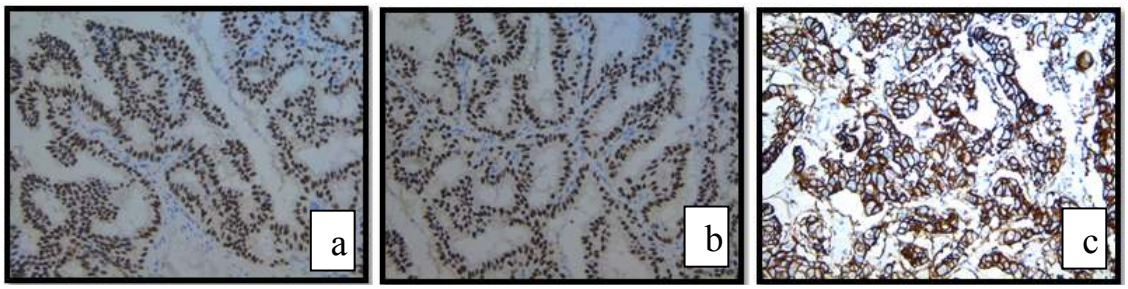
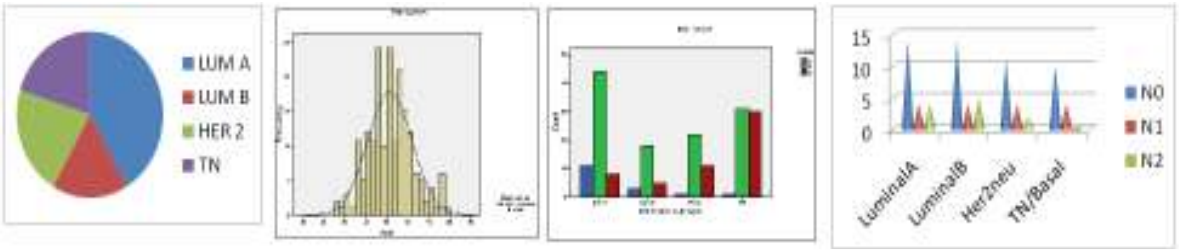


Figure 2



Graph 1 **Graph 2** **Graph 3** **Graph 4**

Legend

Figure 1: Infiltrating ductal carcinoma NOS H and E 200X a) Nottingham grade1, b)Nottingham grade2, c)Nottingham grade3

Figure 2: Immunohistochemistry 200X a) Estrogen Receptor (ER) – Nuclear positivity b)Progesterone Receptor (PR) – Nuclear positivity c)Human epidermal growth factor (Her 2 neu) – Membrane positivity

Graph 1: Number of cases in each molecular subtype

Graph 2: Age distribution

Graph 3: Nottingham grade of tumor

Graph 4: Nodal status

Of the total number of 193 cases, 35% were luminal A,14 % were luminal B,18 % were Her2 and 34 % were triple negative molecular subtype. (Graph No.1) The age of patients ranged from 34 years to 75 years with a mean age of 51.4 years. The highest incidence of breast cancer was between the age group of 51 to 60 years (Graph No.2). The mean age of triple negative cases was 48.7 years. This was much lesser than the other molecular subtypes. This earlier age at presentation of triple negative cases was statistically significant at the level of 0.011. However, the age distribution among the different molecular subtypes was not statistically significant. The mean size of the tumor, among all the molecular subtypes was 3.9cm. T2N0 was the most common stage of presentation among all molecular subtypes. Most of the cases presented at Nottingham grade 2, irrespective of the molecular subtype(Graph No.3). Fifty percent of the triple negative cases, were of grade 2 and 48.4% were of grade 3. This was statistically significant at the 0.002 level.

DISCUSSION

Breast cancer is by far the most frequent cancer among women (21% of all cancers) and is one of the leading causes of cancer deaths in women worldwide. It ranks third overall when both sexes are considered together. It is the most common cancer among women in all the "developed" areas Statistically significant variation in the stage of disease at the time of presentation was noted in different geographic regions and races in a study done in the United States. Women living in urban areas are at a higher risk of developing breast carcinoma compared to rural women. India's National Health Profile 2010 predicted that by 2020, breast cancer will overtake cervical cancer as the most common type of cancer among women in India. (ICMR data) In India the average age of high risk is 43 to 46 years.[8] In our study the age ranged from 34 years to 75 years with a mean age of 51.4 years. The highest number of cases were seen between the age group of 51 to 60 years. In a study by Terfa S.Kene *et al.*, among the Nigerian population, the mean age at presentation was 44.5 +/- 13years.[9] In another study by El-Tamer *et al.* mean age among the African-American patients was 54.17, and 60.35 among Caucasians.[10] Mean age at presentation of triple negative breast cancer was 53years[11] in the study done by Rebecca Dent *et al.* at Canada. Triple negative patients presented at a relatively younger age in our study (48.7 years). The therapeutic modalities and prognosis of breast cancer is influenced by several factors. According to Alison *et al.* prognostic factors[12] for breast cancer include age, tumor size, histologic subtype and grade, axillary lymph node status, lymphatic/vascular invasion, hormone receptor status. The histopathological type of breast cancer is one of the important prognostic factors. In situ cancers (DCIS/LCIS) are slow growing, indolent tumors. The pathological variants with a favourable prognosis are tubular, cribriform, mucinous and adenoid cystic variants, while intermediate prognosis is seen with medullary, secretory and invasive lobular cancers. Infiltrating ductal carcinoma, NOS has a poor prognosis compared to the above mentioned variants. In addition, it is the most common histological type. Eighty percent of breast cancers reported, belong to this type. We also found this histological subtype to be the most common in our study. Therefore we chose to study this subset of breast cancer. Patients in the triple negative category had relatively large tumors as observed by Rebecca Dent *et al.* and Katrina R.Bauer *et al.*, The mean tumor size was 3.1cm, in a study by Andre Albergaria. In our study, most cases presented in T2 stage of breast cancer irrespective of the molecular subtype with a mean tumor size of 3.9cm. In a study by Andre Albergaria *et al.*, the Nottingham grade of triple negative cases were of grade 3. In our study, grade 2 was more common followed by grade 3 among all the molecular subtypes. In a study by Leonel *et al.*, 80% of

cases were N0 stage, 65% were N1, 48% were N2 and 44% were N3. However, on comparing N1 with N2 and N3 disease regardless of tumor size, there were no significant difference in disease free interval or overall survival rates. N0 was the commonest stage of presentation among triple negative cases accounting to 41% in our study. This is in concordance with a study by Andre Albergaria *et al.*. In the triple negative breast cancer cases once there is evidence of lymph node metastasis, other biomarkers do not have a significant role in prognosis as observed by Emad *et al.* The next important parameter which is essential for planning therapy and prediction of prognosis is ER, PR and Her2neu status. According to Adedayo A. Onitilo *et al.* Luminal A[12] is the most common molecular subtype of breast carcinoma. However in our study, Luminal B was the commonest molecular subtype(31%) closely followed by Luminal A(30%). PR expression status has been found to be a more significant prognostic factor than ER expression PR negative patients had a shorter disease free interval than PR positive patients. Jeffrey *et al.* found that trastuzumab therapy achieves excellent results in the treatment of HER-2/neu–positive advanced disease. Extensive evaluation for its potential efficacy when used at earlier clinical stages and the potential role of Her2neu as a predictor of response to therapies for other cancers are being resolved by large prospective clinical outcome studies. In our study, 63 out of a total number of 190, were of triple negative molecular subtype. This accounts to 34% of the total number of cases. This is much higher than the incidence reported incidence of 16.3% by Rakha *et al.* WD Foulks *et al.* state that majority of triple negative cases were grade 3, ductal/no-specific-type carcinomas. There were positive associations with larger size, pushing margins, poorer Nottingham Prognostic Index, development of recurrence and distant metastasis, and poorer outcome. In the lymph node-positive subgroup, both size and androgen receptor retained their prognostic significance. The above findings are concurrent with our study.

CONCLUSION

Luminal A was the most common molecular subtype. Triple negative formed a sizable proportion of cases closely following luminal B. Triple negative patients presented with large tumors and at a younger age compared to the other molecular subtypes.

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Conflict of Interest: None Declared