



Original Research Article

Study of hormone receptor status and HER/2-neu expression in breast malignancies and its implication in molecular subtyping in a tertiary care hospital

Nirmal Daniel¹, Mangala Goneppanavar¹, Sowmya Srinivasan^{1,*}, Anandraj Vaithy K¹

¹Dept. of Pathology, Mahatma Gandhi Medical College & Research Institute, Puducherry, India



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ABSTRACT

Background: Breast cancer has ranked number one cancer among Indian females. In addition to conventional histopathology based on morphology, the College of American Pathologists (CAP) and American Society of Clinical Oncology (ASCO) recommend evaluation of hormone receptors – Estrogen Receptor (ER), Progesterone Receptor (PR) as well as HER2. The role of hormone receptors as a prognostic and therapeutic tool in breast cancer is widely accepted. The molecular subtyping is formulated by immunohistochemical characterization as well as gene expression profiling though the latter is currently not feasible. In the present study we retrospectively measured the frequency of hormone receptor and HER2 positivity in breast cancer patients and classified into the molecular subtypes.

Materials and Methods: We conducted a three year retrospective study on 45 cases of breast cancer who underwent Modified radical mastectomy (MRM), subjected to immunohistochemical evaluation for the status of hormone receptors and HER2 expression as per the ASCO/CAP guidelines. In addition, the clinical details pertaining to patient age, sex, tumour size and histological type were recorded. The molecular subtype of each case was determined and the prevalence compared with similar studies in literature.

Results: The predominant histopathological type in this study was Invasive ductal carcinoma (93.3%). Immunohistochemistry for hormone receptor status revealed ER positivity of 55.5%, PR positivity of 46.6% and HER2 positivity of 33.5%. Among molecular subtyping Luminal A attributed to 33.3% of the cases and was the most prevalent followed by HER2 enriched with 26.6%.

Conclusion: The combined utility of conventional histopathology coupled with immunohistochemical assay based molecular subtyping for routine clinical practice enables diagnosis, estimating prognosis and predicting response to treatment.

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1. Introduction

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. As described in global and Indian studies, there is significant increase in the incidence and cancer-associated morbidity and mortality in the Indian subcontinent.^{1,2} Breast cancer has ranked number one cancer among Indian females with age adjusted rate as high as 25.8 per 100,000 women and mortality of 12.7 per 100,000 women.³ The pre-operative diagnosis of breast

cancer is a multi-disciplinary approach involving detailed and focussed clinical examination, radiological imaging studies and fine needle aspiration cytology. However, the role of conventional histopathology in morphological examination remains the mainstay in diagnosis. The role of histopathologist as a diagnostic oncologist is entitled to determine the biological behaviour of tumour in terms of histological type, extent of differentiation, mitotic activity, microscopic lymphovascular invasion and metastasis.⁴ All this information represents the firm foundation upon which the treatment strategy is built.

While molecular and genetic testing is very elegant, prognostic and predictive, it is still not yet widely available

* Corresponding author.

E-mail address: nirmaldaniel89@gmail.com (S. Srinivasan).

in developing countries like India.⁵ The College of American Pathologists (CAP) and American Society of Clinical Oncology (ASCO) recommend evaluation of hormone receptors – Estrogen Receptor (ER), Progesterone Receptor (PR) as well as HER2 (Human Epidermal growth factor Receptor type 2) for all newly diagnosed cases of carcinoma breast and recurrent cases.⁶ The immunohistochemical (IHC) classification provides both therapeutic and prognostic information and is comparatively less expensive and readily available. IHC based classification of both, ER PR and HER-2/neu status in combination, provides prognostic and therapeutic information which cannot be achieved from either alone.

In this study an attempt is made to determine the molecular subtype of breast cancer based on the IHC characterization.

2. Materials and Methods

The study was undertaken in the Department of Pathology in a tertiary care hospital at Puducherry following approval from the Institutional Human Ethics Committee. Study subjects included breast cancer patients of both sexes with age ranging from 20 to 80 years, who underwent Modified radical mastectomy (MRM) and were reported consecutively during the three year tenure (November 2014 to October 2017). We excluded trucut needle biopsy of breast in view of low cellularity owing to limited tissue sample and resected non-neoplastic lesions of breast. For the 45 cases fulfilling the above criteria ER, PR and HER-2/neu stained slides were retrieved and reviewed as per ASCO / CAP guidelines by the observer using light microscopy after masking the patient identifiers. Based on the immunohistochemical characterization the molecular subtype of each case is derived. The clinical details pertaining to patient age, tumour size, laterality of the breast tumour along with histological subtype were later compiled in a data collection proforma and tabulated as a master chart in Microsoft excel spreadsheet. The positivity of hormone receptor status was expressed as percentage of distribution in tables and graphs. We exercised waiver of informed consent since this was a retrospective study from the tissue archives.

3. Results

The present study includes a total of 45 breast cancer cases diagnosed during the three year study period. The mean age for the study subjects were about 53 years. The tumour size ranged from 3 to 6 cm (mean tumour size 4.7cm). Among the histological types Infiltrating ductal carcinoma attributed to 93.3% (42 cases) and Mucinous carcinoma attributed to 6.34% (3 cases).

Among the study population of 45 cases, maximum incidence of breast cancer was noted in the 6th decade (51

to 60 years) accounting to 16 cases out of which 14 cases showed a histological type of Infiltrating ductal carcinoma while 2 cases were Mucinous carcinoma. Second peak of disease incidence was noted in 5th decade (41 to 50 years) accounting to 11 cases out of which 10 cases were of Infiltrating ductal carcinoma and a case of Mucinous carcinoma (Graph 1).

ER, PR and HER2 receptor status were evaluated by IHC in all cases (Figure 1). Results are as follows (Table 1): Out of 45 breast cancer cases, 21 cases (46.6%) were ER/PR positive, 20 cases (44.4%) were ER/PR negative. None of the cases were identified as ER-/PR+, while ER+/PR- cases were only 4 (8.8%). Out of 45 breast cancer cases, only 16 (33.5%) were positive for HER2. 7 Cases (8.8%) showed equivocal HER2 results. Owing to limited resources, Fluorescence in situ hybridization (FISH) could not be performed for further correlation for the 7 cases with equivocal HER2 (score 2+) results.

With regards to molecular subtyping (Graph 2), Luminal A was the most prevalent subtype constituting 33.3% of the cases, followed by HER2 enriched with 26.6% of cases. Basal-like subtype account to 15.5% of the cases with a similar distribution in unspecified group due to equivocal IHC HER2 score. Luminal B was least frequent with only 8.8% of the cases (Table 2).

Table 1: Results of ER / PR / HER2 receptor status of breast cancers

Parameter	Number	Percentage (%)
ER		
Positive	25	55.5
Negative	20	44.4
PR		
Positive	21	46.6
Negative	24	53.2
Combined hormone receptor sensitivity		
ER+ PR+	21	46.6
ER+ PR-	4	8.8
ER- PR+	0	0
ER- PR-	20	44.4
HER 2		
Positive	16	33.5
Negative	22	57.7
Equivocal	7	8.8

4. Discussion

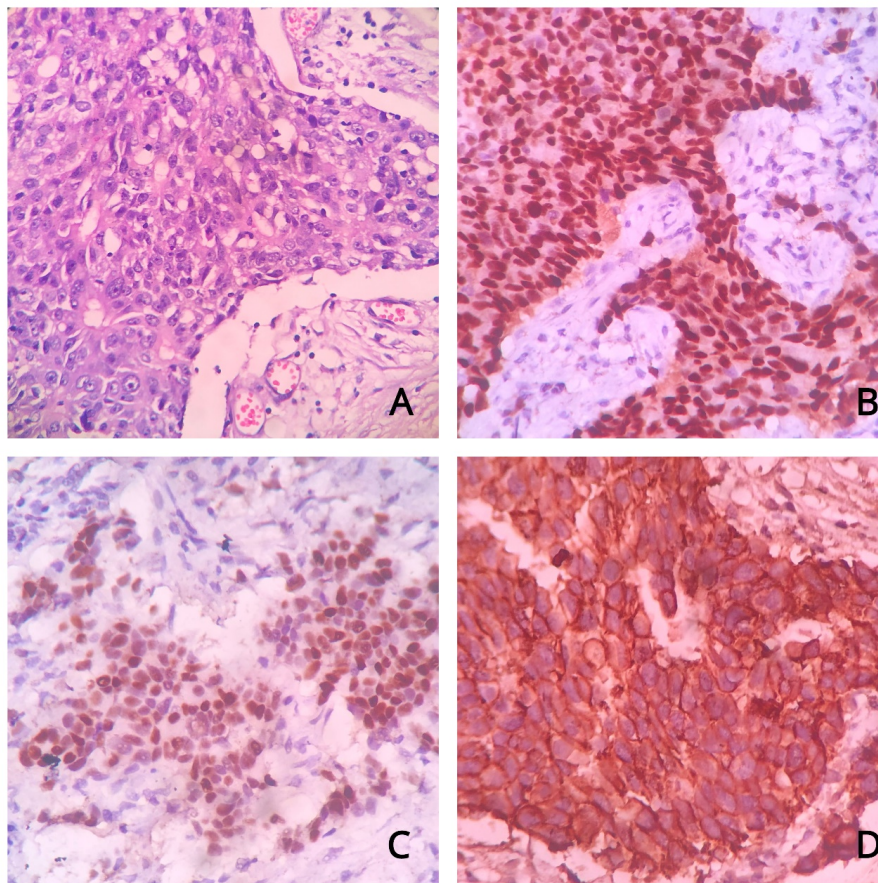
The incidence of breast cancer has increased globally over the last several decades. A retrospective multi-national collaborative study conducted by Agarwal et al., in 2007 estimated over 100,000 new breast cancer cases to be

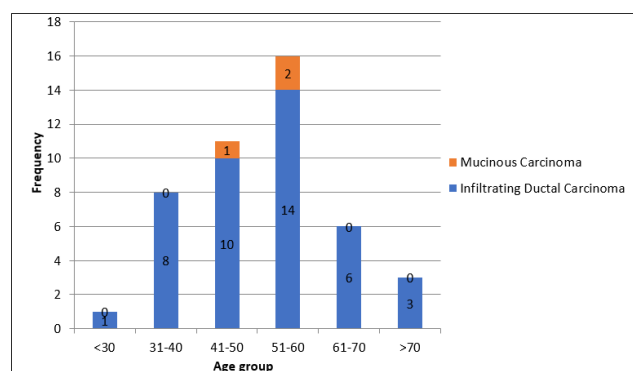
Table 2: Molecular subtypes of breast cancer based on immunohistochemical characterization

Molecular subtype	IHC characterization	Number	Percentage (%)
Luminal A	ER+ PR+ HER2-	15	33.3
Luminal B	ER+ PR+ HER2+	4	8.8
HER2 enriched	ER- PR- HER2+	12	26.6
Basal-like	ER- PR- HER2-	7	15.5
Unclassified	ER± PR± HER2 equivocal	7	15.5

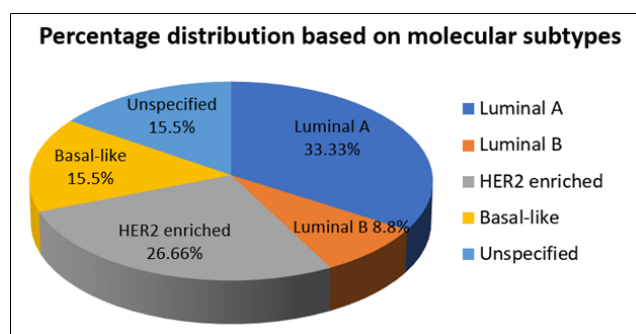
Table 3: Comparison of molecular subtypes with other studies

	Luminal A	Luminal B	HER2 enriched	Basal-like	Unspecified
Fernandes et al (n=134)	35.0%	19.4%	16.4%	29.1%	0
Kumar et al (n=56)	34%	17.8%	17.8%	25%	5.4%
Present study (n=45)	33.3%	8.8%	26.6%	15.5%	15.5%

**Fig. 1:** Invasive ductal carcinoma; **A:** H&E, 40x; **B:** Strong nuclear staining for ER, 40x; **C:** Strong nuclear staining for PR, 40x; **D:** Complete membranous staining for HER2, 40x



Graph 1: Age-wise distribution of histological types among breast cancers



Graph 2: Percentage distribution based on molecular subtype

diagnosed annually in India.⁷ Among the developing countries especially India, the breast cancer cases are expected to have a further rise of 26% by 2020.⁸ In view of the rapidly increasing incidence of the disease among Indian females we undertook the study to derive the molecular subtype of breast cancers based on the hormone receptor status and HER2 expression which is quintessential for planning treatment strategies.

The present study with a study population of 45 breast cancer cases show ER positivity of 55.5% and PR positivity of 44.4%. Few Indian studies have also documented lower positivity for both the receptors. Desai et al. in his study about hormone receptor status of breast cancer in 798 subjects documented a low ER positivity of 32.6% only while PR positivity was seen in 46.1%.⁹ A similar Indian study by Redkar et al. also noted higher incidence of hormone receptor non-reactivity among breast cancer patients.¹⁰ Such results are partially explained by the fact that study group comprises of younger premenopausal women with median age of 48 years presenting with tumours of higher grade. Young women have high levels of circulating oestrogens and a correspondingly low expression of hormone receptors in the tumours.

Muddawa, in a study among 151 breast cancer cases from Sri Lanka documented a prevalence of 45.7% ER positivity and 48.3% PR positivity.¹¹ The patients had high-grade tumours which explains the low prevalence of hormone receptor expression. Among the Asian countries, Chariyalertsak et al. in his study comprising of 83 breast cancer cases in Thailand reported results similar to Desai et al. with 36.1% ER and 45.8% PR reactivity.¹²

The prevalence of hormone receptor-positive breast cancer in Asian countries has been found to be lower than the western world where more than 50% tumours express hormone receptors. Christopher et al. have documented a prevalence of 76-78% of hormone receptor-positive breast cancers in the United States from 1992 to 1998 with a rise in the prevalence over the years.¹³ Barnes et al. among a population of 170 breast cancer patients in London in early 1990s showed 65% of cases to be ER positive.¹⁴ This observation suggests that breast cancer seen in the Indian population may be biologically different from that encountered in Western practice. This can be attributed partially to the dietary, lifestyle and genetic factors among the two ethnic groups which further needs corroborative evidence supported by large cohort studies.

Another interesting finding noted from the present study is that there is no single case demonstrating expression of PR positivity but not ER. Similar observations were made by Kaul et al. in his study among 55 cases of breast cancer in the northern hilly state of Himachal Pradesh, a first of its kind from this region. This study documented low ER and PR positivity of 34.5% and 36.4% respectively.¹⁵ In a study by Hefti et al. which employed gene expression profiling data along with clinical and IHC data across two large and diverse datasets clearly mentioned that ER negative and PR positive breast cancers are not a reproducible subtype. It also mentions that PR expression is not associated with prognosis in ER negative breast cancer.¹⁶ It is important to note that testing for PR expression currently provides no clinically actionable information in ER positive breast cancer, as patients will receive endocrine therapy regardless of PR status and there is no consensus as to whether knowledge of PR expression by IHC has a role in informing the use of chemotherapy in ER positive breast cancer.

With predominantly older and post-menopausal females in the present study, HER2 positivity was observed in 33.5% cases. In a previous study by Dawood et al HER2 positivity was noticed in around 15% cases of carcinoma breast.¹⁷ A five year retrospective study conducted by Siddiqui et al in Northern India has noted a remarkably higher 62% HER2 positivity. The author also claims the result to be attributed to predominantly younger patient population in their study group.¹⁸

4.1. Trends in molecular classification

The prevalence of molecular subgroups (Table 3) in our study were: Luminal A subtype 33.3%, Luminal B 8.8%, HER2/neu 26.6%, Basal 15.5% and Unspecified 15.5%. In a recent study by Fernandes et al on 134 breast cancer cases reported the prevalence in his series as Luminal A 35%, Luminal B 19.4%, Basal 29.1% and HER2/neu 16.4% cases.¹⁹ Another study by Kumar et al. noted similar observations.²⁰ These results are similar to that mentioned in the literature. However, overexpression of the protein and/or amplification of the HER2 gene has been reported in our study as 26.6% (16 out of 45 cases) which is slightly higher as compared to the other studies. All 16 cases were diagnosed as high grade Invasive ductal carcinoma. In this setting, Ki-67 labelling index is necessary to determine the high proliferation rate associated with HER2 enriched subtype. Furthermore, 7 out of 45 cases showed an initial equivocal HER2/neu score but reflex testing with FISH were not performed due to limited resources accounting to 15.5% cases categorised as unspecified.

5. Limitations

As with majority of the studies, design of the current study is subject to limitations owing to smaller sample size. The study population is further limited to mastectomy specimens for which prior history of neoadjuvant chemotherapy are not known. Despite the usefulness of the hormone receptor status there are plentiful variation in the immunohistochemical testing in pre-analytical, analytical and post-analytical levels. The present study yielded an equivocal (score 2+) initial HER-2/neu testing accounting to 15.5% of cases however reflex test with FISH could not be performed in view of limited resources and financial constraints.

6. Future Perspectives

In an era of modern pathology, our present study can further be strengthened with immunohistochemistry by assessing the tumour cell proliferation with a Ki-67 biomarker and comparing it with the proliferative activity in terms of tumour mitosis using histochemical stains. Ki-67, a non-histone protein has been extensively studied as a predictive and prognostic marker in cancers. In the domain of breast cancers, the rate of proliferation is determined by Ki-67 labelling index as low (less than 15%), intermediate (16-30%) and high (more than 30%).

Due to the intrinsic subjectivity of the results encountered in these ancillary techniques, laboratories should employ strict test standardisation and continuously monitoring quality control. Further extensive studies are also required in larger group by taking into consideration clinico-pathological parameters, immunohistochemical findings, lifestyle, genetic influences and genomic assays to

substantiate molecular subtypes for devising personalized treatment strategies. It can only be achieved by close association between pathologists, oncologists with focussed interdepartmental case-based discussions and audit.

7. Conclusion

Based on the results and the methodology employed, we have concluded that Luminal A type was the predominantly observed molecular subtype in terms of prevalence followed by HER2 enriched. Integrating the utility of conventional histochemical stains along with established immunohistochemical and molecular biomarkers for routine clinical practice enables diagnosis, estimating prognosis and predicting response to treatment.

8. Source of Funding

None.

9. Conflict of Interest

None.

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Author biography

Nirmal Daniel, Tutor

Mangala Goneppanavar, Associate Professor

Sowmya Srinivasan, Professor & Head

Anandraj Vaithy K, Associate Professor

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