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Original Research Article

Molecular classification of breast cancer using IHC markers: experience from a tertiary cancer center in south India

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ABSTRACT

Background: Breast cancer is a very heterogeneous disease. Molecular or intrinsic subtypes of breast cancer are based on the gene expression profiling. Doing gene expression profiling in each case is practically difficult. So most of the labs depend on immunohistochemistry to classify breast tumors into various molecular-like subtypes. In this study, we have used immune histochemistry to classify tumors into various subtypes.
Methods: We have retrospectively collected the data of breast cancer patients treated at Apollo Cancer Center, Chennai, in whom ER, PR, HER 2 Neu and Ki 67 were done, and the data was analyzed.
Results: The commonest molecular subtype observed in the present study was Luminal B HER2 positive, constituting 40% of the cases, followed by a HER2 positive (non-luminal) subtype in 20% of cases. The triple negative subtype was the third most frequent, comprising 18% of the cases. The least frequent subtype was Luminal A, seen in only 8% of cases.
Conclusions: There is a higher proportion of luminal B HER2 positive and triple negative subtypes in our study population compared to the other studies in published literature. The proportion of luminal A was lesser in our study compared to the literature.

Keywords: Breast cancer, Immunohistochemistry, Molecular subtypes

INTRODUCTION

Breast cancer does not represent a single disease process. Breast cancer as a disease is classified into different categories based on the histopathological types, grade of tumor, stage of the tumor, and the expression of proteins and genes. Diseases with similar clinicopathological features have dissimilar behaviour and vary in their responses to therapy.[1] The traditional classification systems based on histo-morphology are insufficient to reflect the true biological and clinical heterogeneity observed in breast cancer. Gene expression profiling (GEP) studies provided a molecular explanation for the heterogeneity observed at the clinicopathological level. The molecular classification of breast cancer is based on gene expression and the corresponding receptor status. The molecular or intrinsic subtypes of breast cancer are luminal A (estrogen-receptor and/or progesterone-receptor positive, HER2 negative, low levels of the proliferation marker Ki-67), luminal B(estrogen-receptor and/or progesterone-receptor positive, either HER2 positive or HER2 negative with high levels of Ki-67), HER2 enriched (estrogen-receptor and progesterone-receptor negative, HER2 positive), triple-negative/basal-like breast cancer (estrogen-receptor, progesterone-receptor negative, HER2 negative). The molecular subtypes exhibit distinct, vastly differing biological

behaviour. However, the application of GEP in routine practice is less successful because of the cost, limited access to technology and the need for fresh tumor material.[2,3]

Most of the low-resource centres in India would find molecular profiling unattainable. Hence the alternative of using IHC as a surrogate can be attempted. The European Society of Medical Oncology Clinical Practice guidelines and Kos Z and Dabbs et al have classified breast cancers into five molecular subtypes based on the IHC expression of ER, PR, Her2 and Ki-67 with specified cut off kept for PR and Ki67.[4,1] These subtypes are Luminal A, Luminal B HER2 negative, Luminal B HER2 positive, HER2 enriched and the Triple-negative breast Cancer (TNBC). The objective of his study was to classify our breast cancer cases into these molecular subtypes to assess the prevalence of each subtype in our population. These patients will be followed up for 10 years, and survival characteristics will be analysed at the end of five years and ten years.

METHODS

The design of the present study is retrospective nature. It was designed and conducted in the medical oncology department, Apollo cancer centre, Teynampet, Chennai. The data was collected retrospectively from the records of all female patients diagnosed with Carcinoma Breast during the period of March 2020 to April 2022.

Inclusion criteria

All female patients diagnosed with breast cancer during this period, in whom ER, PR, Her2 and Ki67 were done on either trucut biopsy or surgical specimens, were included. Both treatment naive and post NACT cases were included.

Exclusion criteria

However, IHC was assessed on the initial trucut (before NACT) to avoid the bias of any possible changes in chemotherapy Cases with the missing data set, or the IHC profile was excluded.

A cut-off value of 20% was set for this study to classify the proliferation indices as low or high. This was in accordance with the St. Gallen Consensus 2015. The IHC slides were reviewed, and PR and Ki-67 were rescored as less and more than 20%. The cases were then grouped into the five molecular subtypes. The classification into subtypes was based on the scheme of classification put forward by the European Society of Medical Oncology Clinical Practice Guidelines and studies by Dabbs et al. Invasive cancers of the breast can be classified into five subtypes based on their pattern of IHC expression Luminal A, luminal B HER2 negative, luminal B HER2 positive, HER2 enriched and triple negative (Table 2).

Clinical data were retrieved from archives of the medical records department. The data was entered in Google Forms, and MS Excel was used for analysis. Descriptive statistical tools like mean and standard deviation were used for continuous variables and frequency and percentages were used for categorical variables. Calculated sample size of the study is 95. All patients will be monitored for 10 years to assess the prognosis and survival data. The chi-square test, Fischer exact tests and Kaplan-Meier curves will be used wherever necessary.

RESULTS

During the period, the study population included 123 patients diagnosed with breast cancer. All these patients were categorised into the five molecular subtypes according to the criteria.

Table 1: Patient demographics.

Variable	Number (%)
Sex	
Female	123 (100)
Male	0
Age	
30-39	10 (8)
40-49	19 (15)
50-59	44 (36)
60-69	40 (33)
70-79	8 (6)
80-89	2 (1)

The age range was from 30 to 85 years, with a mean age of 54. Male patients with breast cancer and patients who took treatment from elsewhere were excluded. IHC markers Estrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal Receptor 2 (HER2) and Ki67 were done in all the cases.

Tumor characteristics

The most common histological type was an invasive ductal carcinoma NST constituting 93% of the total cases. Other histological types seen were mucinous carcinoma, invasive ductal carcinoma with mucinous features, invasive lobular carcinoma, neuroendocrine tumor and extensive DCIS with micro-invasive carcinoma. Based on the Nottingham and Modified Bloom Richardson grading system, the most common histological grade was grade 2, comprising 70%. This system takes into consideration the tubule formation in the tumour, nuclear grade and mitotic rate.

ER found to be positive in 59% of the cases, and HER2-positive cases in our series were 24%. Ki67 index was high (>20%) in 59% and 63% of the HER2-enriched and triple-negative breast cancer subtypes, respectively. When observed Ki67 values were compared with the HER2 status. HER2-positive cases showed higher Ki67

proliferation index (45%) than HER2-negative cases (35%) (Table 2).

Table 2: Criteria for molecular classification based on IHC markers.

Subtype	Characteristics
Luminal A-like	ER+, PR>20%, HER2-, Ki67 low (<20%)
Luminal B-like (HER2-)	ER+, PR<20%/, HER2-, Ki67 high (> 20%)
Luminal B-like (HER2+)	ER+, PR any, HER2+, Ki67 any
HER2+(non-luminal)	ER-, PR-, HER2+,.Ki67 any
Triple negative	ER-, PR-, HER2-, Ki67 any

Table 3: Comparison of prevalence of molecular subtypes.

Molecular subtype	Our study (n, %)	Tang et al (%)
Lum A	10 (8.1)	30-40
Lum B HER2-	18 (14.6)	20-30
Lum B HER2+	49 (39.8)	6-10
HER2+	24 (19.5)	12-20
TNBC	22 (17.9)	15

Based on ESMO guidelines, all the cases were classified into molecular subtypes based on the IHC pattern of expression, into luminal A, luminal B HER2 negative, luminal B HER2 positive, HER2 enriched and triple negative subtypes. The commonest molecular subtype observed in the present study was luminal B HER2 positive, constituting 40% of the cases, followed by a HER2 positive (non-luminal) subtype in 20% of cases. The triple negative subtype was the third most frequent, comprising 18% of the cases. The least frequent subtype was luminal A, seen in only 8% of cases (Table 3).

Overall, the most common stages of presentation in each of the T, N and M categories were T2, N1 and M0. In this audit, the tumor stage of T2 was the most common stage at presentation (52%). All the cases of luminal A subtype presented as either T1 or T2 lesions. The majority of the luminal B HER2 negative subtype belonged to T2. HER2 enriched subtype and triple-negative subtype were presented earlier in our study, with T2 being the most common (Table 4).

The most common nodal presentation overall was N1, seen in 42% of cases, followed by N0 in 37%, N2 in 15% and 7% showing N3 status. 58% of the luminal A subtype presented with N0 stage. The triple-negative subtype is presented as the N0 stage in the present study (Table 5).

Table 4: Molecular subtypes and T stage.

Parameters T stage	Molecular classification N (%)					P value
	Luminal B (HER2+)	Non luminal B (HER 2+)	Triple Negative	Luminal B (HER 2-)	Luminal A	
T1	7 (14.3)	8 (33.3)	7 (31.8)	3 (16.7)	3 (30)	0.220
T2	22 (44.9)	11 (45.8)	13 (59.1)	11 (61.1)	7 (70)	
T3	12 (24.5)	4 (16.7)	-	4 (22.2)	-	
T4B	7 (14.3)	1 (4.2)	2 (9.1)	-	-	
T4C	1 (2)	-	-	-	-	

Table 5: Molecular subtypes and N stage.

Parameters N stage	Molecular classification N (%)					P value
	Luminal B (HER2+)	Non luminal B (HER 2+)	Triple Negative	Luminal B (HER 2-)	Luminal A	
N0	18 (36.7)	6 (25)	11 (50)	6 (33.3)	5 (50)	0.140
N1	17 (34.7)	14 (58.3)	10 (45.5)	8 (44.4)	3 (30)	
N2a	11 (22.4)	4 (16.7)	1 (4.5)	3 (16.7)	-	
N3a	3 (6.1)	-	-	-	1 (10)	
N3c	-	-	-	1 (5.6	1 (10)	

DISCUSSION

Breast cancer is the most commonly diagnosed cancer in females in India and the world over. Significant developments have taken place in the past few years in our understanding and classification of breast cancer. The traditional classification systems, which are based on morphology, are insufficient to reflect the true biological and clinical heterogeneity observed in breast cancer.[2,3] Fifteen years have passed since the initial description of intrinsic molecular subtypes in breast cancer.[1] These aspects are slowly gaining acceptance and are being incorporated into routine clinical practice and patient care. The recent developments in molecular aspects have profound implications for therapy and prognostication.

The present study focuses on the classification of breast cancer into molecular subtypes based on the IHC expression of ER, PR, HER2 and Ki67. Based on the ESMO guidelines and inputs from Dabbs et al, criteria based on the presence or absence of IHC expression of ER, PR, HER2 and Ki 67 with a cut-off value of 20% for both PR and Ki-67 used for the classification.(1,4). 123 cases were categorized into the five molecular subtypes based on the adopted criteria. Sixteen cases were excluded as they did not fit into the criteria. The patients ranged from 30 to 85 years of age, with a mean age of 54. Other studies have reported a mean age between 50 and 70 years .[5,6]

The most common histological type reported was invasive carcinoma, no special type, comprising 94% of the cases. The remaining subtypes were invasive lobular carcinoma, mucinous carcinoma and extensive DCIS with micro-invasive carcinoma. This was similar to other reported series.[15] Identifying an exact histological subtype is a known difficulty in cases of needle core biopsies and post-NACT specimens.

Nottingham grade was given in all the cases based on the Modified Bloom Richardson system, which takes into account the features of tubule formation, nuclear grade and mitotic count. The most common grade was Grade 2, comprising 70% of the cases and the next common of Grade 3 (24%). Various studies and published literature on breast cancer grading have shown Grade 2 as the most common finding.[5]

Hormonal receptor ER was positive in 68% and PR in 49% of the cases. Onitilo et al, in their study of breast cancer subtypes based on ER, PR and HER2 expression, reported 78% ER-positive cases and 60% PR-positive cases.[5] In another study by Nadji et al., in 5993 cases of breast cancer, 75% had ER positivity and 55% PR positivity.[9,10]

HER2 assessment was done per the ASCO CAP reporting guidelines.[11] HER was found to be positive in 60% of our cases. Tang et al.'s work on "Immunohistochemical surrogates for Molecular Classification of Breast Carcinoma" reports a HER2 positive rate of 20-30% across various studies.[7] In a separate meta-analysis of different studies among the Indian population, Sandhu et al report a 27% HER2 positive rate.[8] However, we have seen a higher proportion of HER2-positive tumours in our study, reflecting the increased prevalence among the Indian population. The onus should be on quality-maintained tissue processing and IHC staining with adherence to the strict criteria of reporting. Misinterpretation of HER2 expression can lead to very expensive medical treatments as well as exposure of the patient to unnecessary medication with the associated risk on the one hand or missed opportunity to potentially cure a patient on the other.[12]

A high proliferation index (Ki67 >20%) was seen in 42% of cases. This is a pointer indicating more aggressive behaviour. A proliferation index of more than 20% was seen in 59% of the HER2 enriched category and 63% of the triple negative category, supporting the described aggressive biological behaviour of both. Other studies have also shown a higher proliferation rate in HER2-enriched and triple negative categories.[13,14]

All the cases were then classified into the five molecular subtypes using IHC markers as surrogates for molecular subtypes. The most common molecular subtype in the present study was the luminal B HER2 positive subtype constituting 40% (n=49) of the cases. 19% (n=24) belonged to HER2 enriched and 18% (n=22) of cases belonged to the triple negative category. There were 15% (n=18) of cases in the luminal B Her 2 negative category, and luminal A subtype was the least common with only 8% (n=10). In a meta-analysis of molecular subtypes of breast cancer among the western population by Tang et al, the most common subtype was a luminal A in 30-40%, followed by luminal B HER2 negative (20-30%), HER2 enriched (12-20%), triple negative (15%) and luminal B HER2 positive (6-10%).[7]

There is a huge variation in the expression pattern of IHC markers in tumors seen in the Indian population compared to the Western population and, consequently, the proportion of each molecular subtype. Table 3 outlines a comparison between the findings of Tang et al and the present study. Sandhu et al, in their work of meta-analyses of breast cancer in the Indian population, described that there is a comparatively higher proportion of HER2 positive (27%) as well as triple negative (31%) cases in India.[8]

All the patients included in the present study will be monitored, and data will be recorded on each follow-up visit. Survival data will also be analysed at the end of five and ten years. Disease-free, progression-free, and overall survival will be calculated for each molecular subtype.

The strength of this study is the strategic use of IHC as a surrogate for molecular classification. IHC testing is now available in most laboratories, and this practice can be easily applied. The limiting factor is that the validation of the protein expression profile with the gene expression profile could not be done at this time.

The limitations of this study are, it's a single institution study and the histopathology and immunohistochemistry reports are not centrally reviewed.

CONCLUSION

Breast cancer is the most commonly diagnosed cancer in females in India and worldwide. Significant developments have taken place in the past few years in our understanding and classification of breast cancer. The recent developments in molecular aspects have profound

implications for therapy and prognostication. These aspects are slowly gaining acceptance and are being incorporated into routine clinical practice and patient care. Our study classified breast carcinomas into five molecular subtypes based on IHC markers of ER, PR, Her2n and Ki 67 with cut-off values assigned for PR and Ki 67 as 20%. We observed a higher proportion of luminal B HER2 positive and triple negative subtypes compared to the other studies in published literature.

Conversely, the proportion of luminal A was lesser in our study compared to the literature. Overall, HER2 positivity was high in the present study, compared to both the Western and Indian populations. However, it has been noticed that Indian data across various studies reflect this difference in the prevalence pattern of molecular subtypes. Standardization and quality control of IHC is an important step in achieving ideal results, which in turn affects the classification of subtypes. Factors such as ideal tissue fixation, the choice of antibody, and the threshold for interpretation of positive immuno-staining can dramatically affect test accuracy and reproducibility.

Further research is warranted in this field to understand the pathogenesis of specific types of breast cancer, as ethnicity influences the prevalence and behavior of different molecular types of breast Cancer.

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