Molecular Classification of Breast Carcinoma by Immunohistochemical Study

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Abstract

Background and Aims: Breast cancer with morphological classification has limitation with similar clinical and histological features behave differently regarding prognosis and therapy response. Hence the molecular classification has been introduced to predict the clinical outcome. The aim of this study were to classify breast cancer into various molecular subtypes using surrogate IHC biomarkers such as ER, PR, Her2/neu and to find the correlation of each subtype with clinicopathological features.

Materials and Methods: A total one hundred and twelve cases were enrolled in the study. The surgical specimens were evaluated histopathologycally; Suitable block was subjected for immunostain (ER, PR and Her2/neu). Based on their expression status molecular phenotyping was done.

Statistical Analysis Used: All the data were analysed with chi square test by SPSS Statistics Version 23.0. Armonk, NY: IBM Corp software.

Results: The mean age of the patient was 51.11 ± 12 years. Most common histological type was invasive ductal carcinoma, no special type (84.8%). Tumor size with <5 cm (68%) and left laterality (60%) being the most prevalent. Majority of cases were in Grade II and pT2 category. Molecular subtypes had following distribution: Basal like and luminal B were accounted 30% each, while luminal A and Her2/neu enriched were 20% each respectively. There was an association between tumour grade with molecular subtypes and ER, PR receptor expression status with significant *p*-value.

Conclusions: Incorporation of molecular subtyping into traditional histopathological reporting help in better therapeutic management and increases prognostic accuracy. In the current study Basal like presented in advanced stage of their disease.

Keywords: Invasive ductal carcinoma; Luminal subtypes; Prognostic markers.

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Received on 10.02.2020, **Accepted on** 16.03.2020

Introduction

Breast cancer has increase in the incidence which has a negative effect on the health globally and as well as in India.[1] In India the lack of awareness about breast cancer coupled with the lack of screening, causes patients to present at a later stage making the prognosis and survival dismal as compared to the western world.[2,3]

The awareness and screening for breast cancer is the prime modality of reducing the morbidity and mortality. Morphologically identical breast cancers can display divergent clinical outcomes and responses to therapy. However with the introduction of predictive and prognostic markers there has been a major advance in the management of breast cancer.[4] The WHO morphological classification has minimal prognostic and predictive implications. However the further grading and staging of the tumour provide better prognostic markers.[5]

In the timeline of further in understanding of breast cancers, immunohistochemistry (IHC) first allowed us to segregate breast cancers into two main classes: estrogen receptor positive (ER+) and estrogen receptor negative (ER−) followed by progesterone receptor (PR) status which is regulated by estrogen.[6] A decade later, the next step forward was the emergence of nucleic acid in situ hybridization. This led to the identification of two new categories, dependent on whether human epidermal growth factor receptor-2 (HER2) was amplified or not. Breast cancers overexpressed with Her2 are associated with poor prognosis and shows better response to anti Her2 targeted therapy. The Perou[7] and Sorlie et al.[8,9] have identified several intrinsic subtypes of breast cancer based on gene expression analysis such as luminal A, luminal B, normal breast-like, HER2-enriched, and basal-like, with heterogeneous behaviour in the clinical outcomes and responses to therapy.[10]

With recent developments in the field it was identified that Imminohistochemistray being feasible and reproducible, acts as a surrogate for gene expression analysis for further classification.[11] Hence routine IHC evaluations on breast cancer tissue may deliver prognostic significance and information to guide clinical management.[12]

The aim in the current study was to classify morphological subtypes of breast cancer into molecular subtypes with the help of restricted panel of immuno markers and correlate with clinicopathological features.

Materials and Methods

A retrospective study was conducted on an one hundred and twelve cases of invasive breast cancer diagnosed in the Department of Pathology, from October 2014 to August 2016. This study included mastectomy/modified radical mastectomy cases which were proven histopathologicaly and excluded breast biopsies, lumpectomies specimens.

Clinical details were collected from the case file. The Heamtoxylin & Eosin slides were retrieved from the archives of the Department of Pathology and clinic-histomorphological features like age, laterality, histological, tumor size, grade, stage and lymph vascular involvement were analyzed. Bloom Richardson grading system was used for tumor grading and overall College of American Pathologists (CAP) protocol was followed for Histopathological diagnosis and all invasive breast carcinoma were graded from Grade I to Grade III according to Nottingham Histologic Score system. Sections were further subjected to immunohistochemical (IHC) study. Primary antibody Ki-67 (Code-GM001, Mouse Monoclonal Antibody, Pathnsitu) and ER, PR and Her2/neu (Code-EP3, Rabbit Monoclonal Antibody, Pathnsitu) were used. The Polyexcel HRP (non-biotin, micro-polymer based) DAB Detection system was used with adequate positive and negative controls.

Interpretation of IHC

- Scoring of IHC for ER, PR, Her2/neu was done according to the 2014 ASCO/CAP guidelines.

- For ER, PR staining score was considered Positive if ≥1% Immunoreactive tumor cells present; Negative if <1% Immunoreactive tumor cells present

- Her2/neu staining was scored as

- 0 = no staining or incomplete, faint/ barely perceptible membrane staining in < 10% of invasive tumour cells;

- 1+ = incomplete, faint/ barely perceptible membrane staining in > 10% of tumour cells;

- 2+ = incomplete and weak-to-moderate circumferential membrane staining of > 10% of tumour cells or complete, intense, circumferential membrane staining in <10% of tumor cells ;

- 3+ = complete, intense, circumferential staining of > 10% of tumour cells.

- Scores of 0 or 1+ was considered tumour negative for Her2/neu expression, score of 2+ as equivocal and required confirmation with FISH, while 3+ was regarded as positive expression of Her2/neu.

- The slides were observed and scored by two independent observers with consensus reached in case of any discrepancy.

Ethical Clearance

The study was approved by institutional ethical committee**.**

Statistical Analysis

The data were analyzed using IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. Frequencies and percentages of all the variables were computed. Chi-square test was used to compare the association of ER, PR, HER-2 expression and molecular subtypes with clinicopathological parameter such as age, tumour laterality, tumor morphological/histological type, tumor size, grade, TNM stage, LVI. The results were considered statistically significant if *p*-value was <0.05

Results

A total one hundred and twelve cases were enrolled in the study. The mean age of patient at the time of diagnosis was found to be 51.11 ± 12 (range from 22–85 years old). The age groups were grouped into three categories as <40, 41–50, >51 with following distribution of cases 20.5%, 31.3%, and 48.2% respectively. All patients were female and majority of them showed left breast involvement.

Most of them (84.8%) were invasive ductal carcinoma IDC-NST (Fig. 1), among them (25.9%) cases were associated with DCIS (ductal carcinoma in situ component). The another 17 cases were categorized as Other subtypes due to there small in number; four cases of mixed

(IDC + Pagets disease), four cases of mucinous and four medullary, three papillary, one each apocrine, and sever anaplastic (Table 1).

Based on ER (Fig. 2), PR (Fig. 3) and Her2/neu expression status (Fig. 4), molecular subtypes are as follows: Basal like and Luminal B were accounted for an about 30.4% each; followed by Luminal A (19.6%) and Her2/neu (19.6%). In all molecular subtype, invasive ductal carcinoma was most common histological type (Table 1).

Age group categories did not find any significant association neither with hormonal status nor molecular subtypes.

Most of our cases (68%) had tumour size <5 cm. There was no significant association between ER, PR, Her2/neu status and molecular subtypes. According to Bloom Richardson grading system; majority of our cases (51%) were in Grade II. We found a significant association between receptor expression status and Luminal subtypes with tumour grading.

Lymphovascular invasion was seen in 60% of cases and there was no significant association with neither hormonal receptor expression status nor molecular subtypes (Table 1). Overall majority of our cases 54% were in Stage 2 (pTNM).

No significant association with neither hormonal receptor status nor molecular subtypes (Table 1).

Table 1: Correlation of Luminal subtypes with variable like age, tumor site, size, grade, pT category and lymphovascular invasion

Variables	Luminal A (*n* = 22)	Luminal B (*n* = 34)	Her2/neu enriched (*n* = 22)	Basal like (*n* = 34)	*p*-value
Age					
≤ 40 yrs (*n* = 23)	6 (26.1%)	5 (21.7%)	4 (17.4%)	8 (34.8%)	0.798
41–50 yrs (*n* = 35)	7 (20.0%)	9 (25.7%)	7 (20.0%)	12 (34.3%)	
>50 yrs (*n* = 54)	9 (19.6%)	20 (30.4%)	11 (19.6%)	14 (30.4%)	
Size					
<5 cm (*n* = 76)	17 (22.4%)	21 (27.6%)	14 (18.4%)	24 (31.6%)	0.622
>5 cm (*n* = 36)	5 (13.9%)	13 (36.1%)	8 (22.2%)	10 (27.8%)	
Site					
Right (*n* = 44)	9 (20.5%)	11 (25.0%)	9 (20.5%)	15 (34.1%)	0.785
Left (*n* = 68)	13 (19.1%)	23 (33.8%)	13 (19.1%)	19 (27.8%)	
Grade					
Grade I (*n* = 19)	6 (31.6%)	8 (42.1%)	1 (5.3%)	4 (21.1%)	0.044*
Grade II (*n* = 57)	12 (21.1%)	20 (35.1%)	10 (17.5%)	15 (25.3%)	
Grade III (*n* = 36)	4 (11.1%)	6 (16.7%)	11 (30.6%)	15 (41.7%)	

Variables	Luminal A (n = 22)	Luminal B (n = 34)	Her2/neu enriched (n = 22)	Basal like (n = 34)	p-value
pTCategory					
pT1 (n = 21)	06 (28.6%)	6 (28.6%)	5 (23.8%)	4 (19.0%)	p = 0.579
pT2 (n = 61)	10 (16.4%)	19 (31.1%)	9 (14.8%)	23 (37.7%)	
pT3 (n = 26)	05 (19.2%)	8 (30.8%)	6 (23.1%)	07 (26.9%)	
pT4 (n = 4)	01 (25.0%)	01 (25.0%)	2 (50%)	00 (00%)	
Lymphovascular invasion					
Present (n = 67)	14 (20.9%)	22 (32.8%)	11 (16.4%)	20 (29.9%)	0.713
Absent (n = 45)	8 (17.8%)	12 (26.7%)	11 (24.4)	14 (31.1%)	

*p < 0.05, indicates statically significance
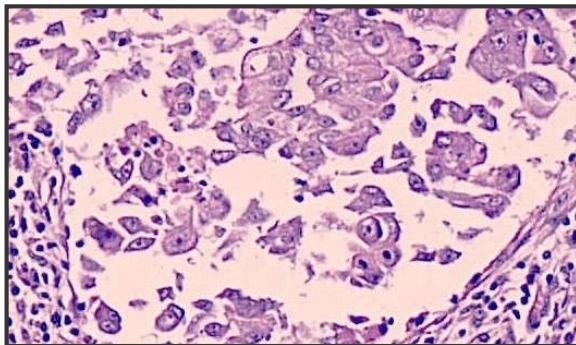

Fig. 1: Photomicrograph of invasive ductal carcinoma (H&E, X40).
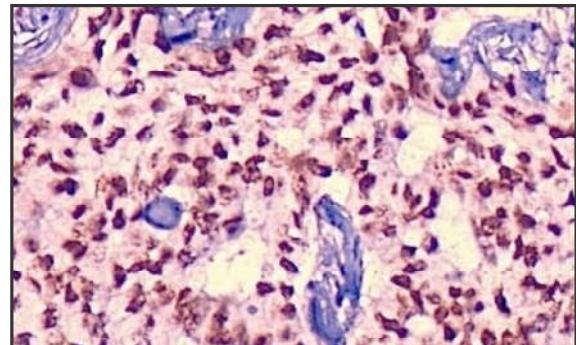

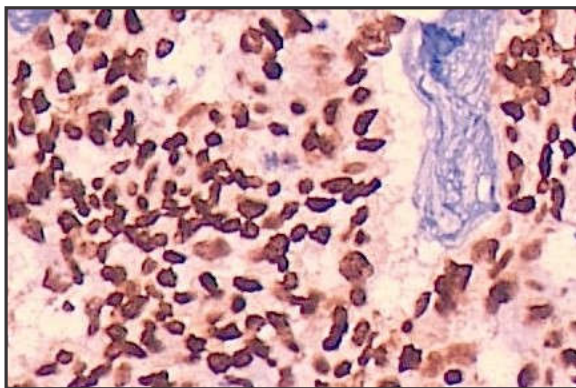**Fig. 2:** Photomicrograph of positive ER immunostain in IDC (ER,X 40).

Fig. 3: Photomicrograph of positive PR immunostain in IDC (PR,X 40).
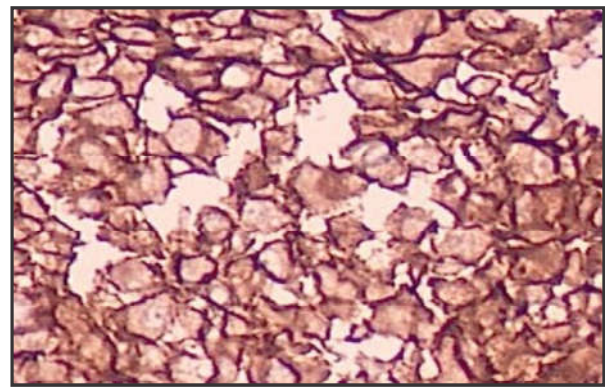

Fig. 4: Photomicrograph of Her2/neu 3+ immunostain in IDC (Her2/neu,X 10)

Discussion

It is well known that breast tumor prediction and prognosis relay on factors such as clinocapthological parameter and IHC expression status. Immunohistochemistry is most important factor in prognosis and hence it has become an integral part in histopathology reports for appropriate treatment.[13] The current study was undertaken to understand the correlation between clinicopathological parameters and IHC profile.

In the present study we had one hundred and twelve mastectomy cases. The age range was between 22–85 years with mean age being 51.11 ± 12 and maximum number of cases were noted in >51 years and found to be concordant with study done by Kanakarajan A et al.[14] in 2015. In an article published by Nikhra P et al.,[15] in 2014 found an age range between 31–75 years with mean age of 49.2. Another study by Rao C et al.[16] in 2013, JCRT noticed age range of 41–50 years with mean age 46.8%. Our study did not find any significant

association between age grouping with hormonal receptor expression status and molecular subtypes.

Among histological types we found IDC-NST was most common type 84.9% with similar findings in other studies done by Nikhra Pet al.,[15] Akbar M[11] and Tiwari S et al.[12] Based on hormonal receptor expression status, molecular subtyping was done and we had found luminal B and Basal like each 30.4% followed by luminal A (19.6%) and Her2/neu (19.6%) subtypes. various other studies had variable distribution (Table 2). Laterality of breast tumor: most frequent involvement being the left side of the breast as compared to right side and which was similar to the study done by Tiwari S

et al.[12] and Ambroise et al.,[18] Azizun-Nisa et al.[19] We discovered that majority of our cases were <5 cm (60%) in tumour size; there was no evidence of significant association of tumor size with hormonal expression status nor with molecular subtypes; which was concordant with Widodo I et al.,[17] were as Rao C et al.,[16] and Spitale A et al.[20] found a significant association between hormonal receptor status and tumor size. Regarding tumour grade we had majority of our cases in Grade II and found a significant association with respect to hormonal receptor status and molecular subtypes, similar finding were observed by Tiwari S et al.,[12] Widodo I et al.[17] and Rao C et al.[16]

Table 2: Proportion of luminal subtypes among different studies

Author	Luminal A	Luminal B	Her2/neu	Basal like
Andrade A C et al.[13]	23.7%	44.6%	14.50%	17.10%
Widodo I et al.[17]	54.8%	16.7%	20.2%	25.0%
Akbar M et al.[11]	28.4%	25.0%	30.0%	16.6%
Kanakarajan A et al.[14]	35.0%	14.0%	18.0%	34.0%
Tiwari S et al.[12]	27.1%	25.7%	25.7%	15.7%
Current study	19.6%	30.4%	19.6%	30.4%

In the current study TNM stage; T2 (54%) was most prevalent followed by T3 (23%), T1 (19%) and T4 (4%) with no significant association between hormonal receptor status expression nor molecular subtypes. Were as Tiwari et al.[12] study found majority of cases in T3 (57.2%) followed by T2 (41.4%) and T1 stage. While Akbar M et al.[11] had following presentation T1 and T2 (38.4%), T3 (45%), and T4 (13.3%). Spitale A[13] had predominance of T1 (42.9%) followed by T2, T3 and T4 none of these studies found any significant association with molecular subtypes. At about 60% of cases had lymphovascular invasion without any significant association with hormonal receptor status or molecular subtypes. Were has Rao et al.[16] had only 19.8% of cases had LVI without significant association. Another study Spitale A[20] had 85.3% positive nodal status with no significant association. While Widodo I et al.[17] found a significant association with respective of nodal status.

Conclusion

Carcinoma of breast are usually manifested in late stage mainly due to lack of awareness and delay in detection. There is a need for breast cancer screening, use of various tests and methodologies (ancillary

studies-IHC) to explore and predict the biology of the cancer which allows the clinician to better understand the biology of tumour and highlight the clinical outcome. Hence the key to treating the breast tumour necessitates its early detection. The current study suggest IHC based assay to identify the main tumor intrinsic subtypes which varies in their clinic-pathological parameters and extremely important to classify into different molecular subtypes which will lead to different prognosis and therapeutic options. Our study demonstrates IDC-NST as the most prevalent histological type and among molecular phenotype, Basal like and luminal B was the most common followed by Luminal A and Her2/neu enriched. Majority of cases were in Grade II and pT2 in pT category. Luminal groups respond to hormonal treatment while her-2/ neu group respond well to biological therapies using transtuzumab. On the other hand, Basal like phenotype, has been associated with poor clinical outcomes, which likely reflect this subtypes have high proliferative capacity as well as the lack of directed therapies since basal-like tumors do not typically express ER or Her2/neu, they usually respond to chemotherapy.

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