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Original Article

Molecular Subtypes of Breast Cancer by Immunohistochemical Profiling

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Abstract:
Objective: To evaluate the frequency of molecular sub types of breast cancer on core needle biopsy and to correlate the subtypes with these clinico-pathologic parameters: age of the patient, histologic type and grade of cancer and lymph-vascular invasion (LVI).
Methods: A cross-sectional, observational study, conducted at Dow University of Health Sciences, Karachi, from December 2014 to December 2015. It included core needle biopsies of 285 patients of breast cancer. Immunohistochemical staining with antibodies for Estrogen Receptor (ER), Progesterone Receptor (PR) and Human Epidermal Growth factor 2 (Her 2) was performed and breast cancers were classified into four molecular subtypes: Luminal A (ER/PR +, HER2-), Luminal B (ER/PR +, HER2+), Triple Negative Breast cancer (TNBC) (ER/PR -, HER2-) and HER 2 (ER/PR -, HER2+). Clinical parameters were compared using chi-square test.
Results: 285 cases were included in this study. The mean age of the patients was 43.3 years (17-88). The frequency of the molecular subtypes of breast cancers was Luminal B 139(48.77%), Luminal A 60(21.05%), Her2 54(18.94%) and Triple Negative Breast cancer 32(11.22%). The most common diagnosis of breast cancer was Invasive Ductal Carcinoma 258 (90.52%) and grade II 230 (80.70 %). There was significant association of molecular subtype of breast cancer with the grade of tumor ($p<0.001$) and with lympho-vascular invasion ($p<0.011$). Her 2 cancers showed the highest frequency of grade 3 and Triple Negative Breast cancer had the highest frequency of lymph-vascular invasion.
Conclusion: Luminal B is the most common molecular subtype of breast cancers in our population. The mean age of breast cancer was younger than most studies. We recommend that the molecular subtyping of breast cancers using immunohistochemistry should be incorporated into histopathology reporting of core needle biopsies, as this may facilitate the clinicians in selection of treatment for the patients.
Key words: Breast cancer, molecular subtypes, receptor status, hormone receptors, immunohistochemistry, Triple negative breast cancer, ER, PR, HER2, HER2/neu

Introduction

Breast cancer is the most common cancer in females with a reported incidence of 1.67 million in 2012.[1] The mortality rate is highest in the females in less developed countries, and a proportionate mortality of 14% of total malignancies. Breast cancer is the most frequent cancer in women in Karachi, accounting for one-third of the cancers in the females and its incidence is second highest in Asia after Israel.[1]

Breast cancer encompasses a heterogeneous group of tumors with great variability at molecular and morphological levels.

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Recent World Health Organization (WHO) classification of invasive breast carcinoma classifies it into more than 21 different morphological subtypes, each with different biological behavior.[1]

Core needle biopsy (CNB) is the standard procedure for the diagnosis of breast cancer in patients at some centers; and has largely replaced the fine needle aspiration cytology (FNAC) and excision biopsies.[1] A pathologist can precisely comment on the morphologic type, grade of breast cancer and lymph-vascular invasion on CNB.[1] Luminal keratin CK 8/18 and myoepithelial markers can be used to distinguish in situ and invasive cancers.[1] Prognostic breast markers: Estrogen Receptor (ER), progesterone receptor (PR), and Human Epidermal Growth-factor Receptor-2 (HER2), are routinely assessed on CNB by Immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH).[1-26]

In 2000, Perou and Sorlie using Gene Expression Profiling (GEP), DNA microarrays analyzed 65 breast tumors for 8102 genes. They pioneered a molecular classification of breast tumors and identified 5 distinct subtypes of breast cancer: luminal A, luminal B, HER2-enriched, Basal-like and normal-like, each with differing clinical outcomes and responses to neoadjuvant chemotherapy, however, use of GEP is not economical and practical in routine diagnostics, therefore, each of these five molecular subtypes are mapped by immunohistochemistry except the normal-like, which shares a similar immunohistochemical status with Luminal A and a molecular profile resembling normal breast. Therefore, using IHC four molecular subtypes can be determined which are:

Luminal A: ER and/or PR positive, HER2 negative

Luminal B: ER and/or PR positive, HER2 positive

Triple negative: ER, PR and HER2 negative

HER2 overexpressed: EP and PR negative, HER2 positive.[1-4]

Very few studies are available from Pakistan on molecular sub typing of breast cancer.[1,2] The objectives of this study were to determine the frequency of different molecular subtypes of breast cancer on trucut needle biopsies using immunohistochemical staining and to correlate each subtype with these clinic-pathologic parameters: age of the patient, histologic type, grade and lymph-vascular invasion (LVI) in breast cancer.

Methodology

This was a cross-sectional, observational study, conducted at Dow Diagnostic Research and Reference Laboratory, Dow University of Health Sciences, Karachi, from December 2014 to December 2015. Core biopsies of 285 cases of primary breast cancer which were received in the department with complete history and request for ER, PR and HER2 tests were included in the study. All specimens received without formalin, mastectomies, lumpectomies, incision and wedge biopsies, all non-epithelial tumors and post-chemotherapy patients with suspicion of recurrence were excluded.

Histological typing of Breast cancer was performed according to WHO classification and grading was performed according to Modified Bloom-Richardson grading system.[3] Immunohistochemical stains ER, PR and HER2 were performed and the breast cancers were classified into 4 molecular subtypes. These subtypes were correlated with the age of the patient, histological type and grade of cancer and lymph-vascular invasion (LVI).

Immunohistochemistry (IHC) was performed on 4 mm thick sections of the tumor, using DAKO envision system. A semi-quantitative score was used to record results of ER and PR staining according to the Allred system which considers proportion and intensity of the stained tumor cells.[4]

Her-2/neu was scored on a 0 to 3 scale according to the guidelines of American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP).[4]

0 and 1 were taken as negative, 3 as positive, and 2 as equivocal and were referred for Florescence In situ Hybridization (FISH). Lympho-vascular invasion **(**LVI) was assessed on H& E slides and was defined as carcinoma cells present within a definite endothelial-lined space.[4] IHC marker CD34 was used to confirm LVI in doubtful cases, which stains the endothelial cells.

Data was analyzed using SPSS version 16. Descriptive statistics were calculated as mean and median for age of the patients and frequency and percentages for the molecular sub types of breast carcinoma. Chi-square test was applied for correlation of molecular sub types of breast carcinoma with prognostic variables including age, tumor type, tumor grade, LVI, ER, PR, HER2 expression. Data was expressed as percentages. A p value<0.05 was considered as to be significant.

Results

Total 285 cases were included in this study. Mean age was 43.3 years (17-88). There were 280 (98.24%) females and 5 (1.75%) males in the study, of these 270(94.73%) were married. Left side of the breast was slightly predominant 145 (50.87%).

The most common histopathological type of breast cancer was Invasive Ductal Carcinoma 258 (90.52%) (Table 1). Most of these cancers were grade II 230 (80.70 %). Frequencies of breast cancers which were ER positive were 193 (67.71%), PR positive 173 (60.70%) & Her 2 positive 193 (67.71%). (Figure 1)

The most common grade among all subtypes was grade II with highest frequency in Luminal B (n=121) whereas, grade I was least common. We found significant association between the molecular subtype and grade of tumor (p=0.001), with highest frequency of Grade 3 in HER2 cancers. There was also significant association between molecular subtype and LVI (p=0.011), highest frequency of LVI was found in

TNBC. There is no association between the molecular subtype and age (p=0.208) or between molecular subtype and type of tumor (p=0.361). Table 3.

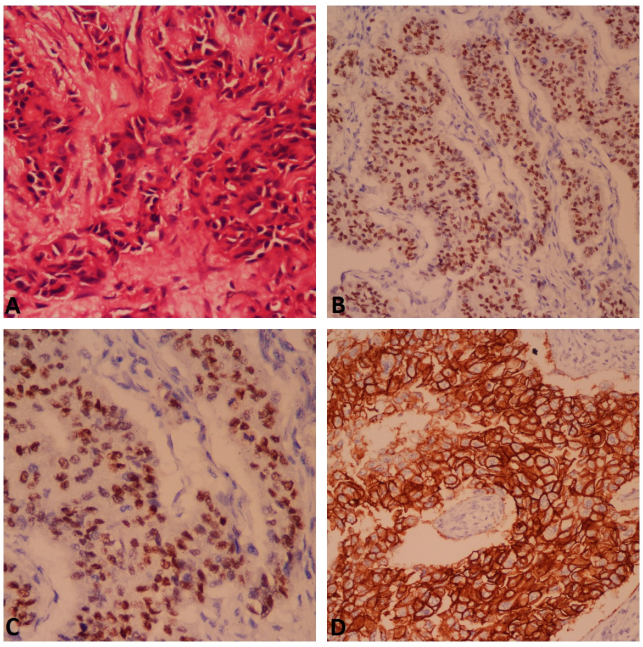

Figure 1; A. H&E Stain of Luminal B carcinoma X 400, B; Strong Nuclear Staining of ER IHC stain X 400, C Strong Nuclear Staining of PR IHC stain X 400.D; Membranous Her2neu staining X400

TABLE 1: Morphological type of breast cancer and their relative frequencies (n=285).

Morphological type of cancer	Number of the cases	Percentage of cases
Invasive Ductal carcinoma	258	90.52%
Invasive Lobular carcinoma	14	4.91%
Metaplastic carcinoma	9	3.15%
Mucinous carcinoma	4	1.40%

Molecular subtype Luminal B breast cancers was the most frequent 139(48.77%), followed by luminal A 60 (21.05%), Her2 54 (18.94%) and TNBC 32 (11.23%). Table2.

TABLE 2: Molecular sub types of breast cancer based on immunohistochemistry

Molecular sub type	Number of cases	% of molecular sub type
Luminal A (ER/PR+,Her2-)	60	21.05%
Luminal B (ER/PR+,Her2+)	139	48.77%

Table 3: Correlation of molecular sub types of breast cancer with clinicopathologic parameters.

Parameters		Luminal A	Luminal B	Her 2	TNBC	p-value
Age	≤50	47(78.33%)	105(75.54%)	38(70.37%)	19(59.38%)	0.208
	>50	13(21.66%)	34(24.46%)	16(29.62%)	13(40.62%)	
Type of tumor	IDC	53(88.33%)	126(90.65%)	50(92.59%)	29(90.63%)	0.361
	ILC	3(5.0%)	6(4.31%)	3(5.56%)	2(6.25%)	
	Others	4(6.67%)	7(5.04%)	1(1.85%)	1(3.12%)	
Grade of tumor	Grade I	0(0.0%)	2(1.44%)	0(0.0%)	0(0.0%)	0.001
	Grade II	52(86.66%)	121(87.05%)	34(63.0%)	23(71.88%)	
	Grade III	8(13.33%)	16(11.51%)	20(37.0%)	9(28.12%)	
LVI	Present	12(20.0%)	16(11.51%)	13(24.07%)	11(34.38%)	0.011
	Absent	48(80.0%)	123(88.49%)	41(75.92%)	21(65.62%)	

IDC: Invasive Ductal carcinoma, ILC: Invasive Lobular carcinoma, LVI: Lymph-vascular invasion.

Discussion

Breast cancers are grouped into at least five "intrinsic" subtypes based on GEP: Luminal A. Luminal B, Her-2 neu, Basal-like and breast-like.[7] Using IHC as surrogate for GEP many different classifications have evolved that divide breast cancer into basal and non-basal subtypes. Basal type tumors are high grade tumors which are usually represented as triple (ER, PR and HER2) negative, positive for basal Cytokeratins CKs 14,19 and 5/6, EGFR, p53. Non-basal tumors are Luminal tumors that are Hormone Receptors (HR), Estrogen & Progesterone, positive; HER2 positive or negative, luminal keratin CK 8/18 positive and are divided into A and B subtypes, defined differently in different classifications. [10] Tang et al., compared 4 different IHC classifications of breast cancers and showed that although these classifications have similar terminologies but these are not interchangeable. [10]

In our study we used ER/ HER2 classification.[8,9,10,11] In our study breast cancer luminal type were 199 (69.82%): Luminal B 139 (48.77%), luminal A 60 (21.05%). Basal type was 86 (30.17%): Her2 54 (18.94%) and Triple Negative Breast cancer (TNBC) 32 (11.22%). Studies show that the molecular subtypes of breast cancer vary from population to population, confirming the molecular heterogeneity and variation in genetic makeup. Our finding of luminal B

predominance is in line with that of Hashmi et al. who determined 69% of their cases to be Luminal B and 31% to be luminal A.[13] A study from Morocco comparing breast cancers in Arabic and European women showed that the Arabic women had a higher percentage of Luminal B than Luminal A (B: 63%, A: 18%) compared to European women (B: 42%, A: 41 %).[3] Most of the studies worldwide show Luminal A to be the predominant subtype. In Algeria percentage of the luminal A, TNBC, luminal B and HER2+ breast cancer subtypes were 50.59%, 20.80%, 19.67% and 8.92%, respectively.[10] Study from Saudi Arabia shows Luminal A to be the most prevalent followed by Luminal B (47% and 27.8%), TNBC 18.3% and HER2 6.9%. [i] Study from Japan shows Luminal A to be 65%, followed by HER2 type 12.5% and Luminal B8.7% and TNBC 7.9%.[4] A population-based study from USA shows Luminal A cancers to be 72.7% followed by TNBC 12.2%, Luminal B 10.3% and HER2 4.6%.[4] Population based study from France shows Luminal A to be 66.8%, TNBC9.2%, Luminal B6.3% and HER2 3.7%.[4]

Compared to Luminal A, Luminal B is associated with younger age of the patient, higher grade and higher frequency of node metastasis.[13] Luminal A tumors require anti endocrine therapy only, whereas adjuvant chemotherapy may be considered in luminal B with high risk for recurrence.[ii]HER2 tumors compared to TNBC, are associated with better response to targeted therapy Trastuzumab (Herceptin) and anthracycline/taxane based chemotherapy. TNBC are treated with anthracycline/taxane based neoadjuvant chemotherapy. Latest St. Gallen consensus meeting 2017 recommends that immunohistochemical stains be routinely used to distinguish Luminal A and B like tumors and that multigene testing like Mamma Print and Onco-type DX may provide useful information. [23]

In our study the IHC molecular classification, grading, typing and LVI were determined on core breast biopsy. According to studies core biopsy is a reliable tool for assessment of molecular subtypes and grade of the tumor. [5] In this era of molecular classification, histopathological grade is still a strong prognostic factor and reduced long-term survival is associated with higher grade of the tumor.[5] Grade 2 was the most common grade assigned to all the subtypes in our study, but statistically significant number of HER2 & TNBC were associated with grade 3 (p0.001). LVI is an independent prognostic factor for local and distant recurrence and is associated with poor disease survival and high rate of node metastasis.[1] The highest percentage of LVI was seen in TNBC in this study (p0.011), which is comparable to the published data indicating that TNBCs are more frequently associated with LVI.[6]

The mean age of breast cancer in our study population was 43 years, younger age of breast cancer has also been reported in some African countries like Algeria, Morocco (47& 48.5 years respectively). This is at least a decade younger than the age class of 65-75 years in US population and 50-60 years in French populations. [21,22]

The strength of the study was that we performed typing, grading of tumor, assessment of LVI and molecular subtyping of breast cancers on core biopsy, which is a minor day care procedure and will help the oncologists in deciding treatment for the patients. The limitations of the study are that it is a not a population-based study. IHC stain Ki67 was not used to differentiate between Luminal A and B subtypes. Limitations of IHC are that there is lack of standardization: in the molecular classifications, in the use of IHC markers and their cutoff points.[iii]

Many new markers including p53, Androgen receptor (AR), p16, Folate Receptor A (FRA) are being explored as new targets for personalized treatment. [26] We recommend investigation in large cohorts with advanced genetic techniques and survival studies to determine the course of this heterogeneous disease.

Conclusion

We report that Luminal B was the most common molecular subtype in our population. The mean age of breast cancer was younger than most studies. We recommend that the molecular subtyping of breast cancers using immunohistochemistry should be incorporated into histopathology reporting of core needle biopsies, as this may facilitate the clinician in selection of optimal treatment for the patients.

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E. Critical Review
F. Facilitated for Reagents/Material/Analysis
G.