Original Article

DOI: 10.21276/APALM.3064

Molecular Subtyping of Invasive Breast Carcinoma by Immunohistochemistry and Five-Year Survival Study

Raja S Vipparla, Raji T Naidu, Susan Cherian* and Suresh S Shettigar

Pathology Department, BARC Hospital

ABSTRACT

Background: Global gene expression profiling for Invasive breast carcinoma (IBC) has identified intrinsic subtypes of IBC with differing clinical outcomes and response to therapy. As genotyping assays are limited by availability and cost, we have used Immunohistochemistry (IHC) surrogates to classify IBC into molecular subtypes.

Methods: Representative tumor blocks of 158 surgical specimens of IBC between 2007 to 2017, were selected and IHC done for ER, PR, Her2, Ki67, CK 5/6 and EGFR. The cases were classified into 7 Molecular subtypes (Luminal A, Luminal B, Luminal HER2PR+, Luminal HER2PR-, HER2Enriched, Basal like (BLBC) and non classifiable (NCBC) and correlated with clinico-pathological findings. Five- year survival rate was calculated for patients diagnosed between 2007 to 2013.

Result: The most common subtype was Luminal A (31.0%), followed by Luminal B (25.3%), NCBC (14.6%) and HER2 enriched (13.3%). Among post-menopausal women, common subtypes were Luminal A (33.8%) and Luminal B (24.4%). Among premenopausal women, most cases were NCBC (27.8%) and BLBC (22.2%). 61.2% of Luminal A were Grade2 and 22.4% were Grade 1. Many cases of Luminal B and HER2 positive cases were of Grade3 (45.0%) and (57.1%) respectively. Of the triple negative category (BLBC & NCBC), 73% were Grade3 with statistically significant correlation (p value < 0.001). Most of these cases were in Tumor stage T2 (70%), followed by T1 (22.3%). Nodal metastasis was seen in 39.6% and 65% respectively of Luminal A and Luminal B subtypes. Distant metastases on follow-up were present in 15.8%, which included HER2 enriched subtype (28.5%), followed by BLBC (20.0%) and NCBC (17.4%). Luminal A cases, had better survival accounting for 88% of all survivors.

Conclusion: Molecular subtyping of IBC using IHC was useful to understand the clinicopathological distinctiveness of each subtype.

Keywords: *Breast Carcinoma, ER, PR, HER-2/neu, Biomarkers*

Introduction

Breast cancer is the most common cancer among women both in developed and developing countries. Female breast cancer ranks as the fifth leading cause of death (6,27,000 deaths per year i.e., 6.6% of total deaths globally due to cancer).[1] Breast cancer in women in India accounts for 14% of all cancers. [2,3] The incidence rates in India begin to rise in the early thirties and peak at ages 50-64 years. Overall, 1 in 28 women is likely to develop breast cancer during her lifetime.[4]

Molecular classification is becoming the gold standard for complete characterization of breast cancer and the underlying technology has already generated gene-profiling models to predict outcomes. [5,6] In 2013, IHC based molecular classification (MC) was recommended in the St Gallen guidelines for clinical decision making.[7] Studies in literature have shown that molecular differences correlate with clinical features, such as survival, prognosis, and treatment sensitivity. The present study is performed to identify molecularly distinct subtypes of breast cancer and to study their prognostic and predictive value and whether routine use of this classification can be done to predict distinct clinical outcomes. There are sparse studies addressing population-based distribution and survival of molecular subtypes in India.

Like many other researchers, we have used IHC surrogates to classify invasive breast carcinomas in to various molecular subtypes, using IHC surrogates which include biomarkers of the estrogen receptor (ER), progesterone receptor (PR)and HER2 and other markers i.e. Ki67, EGFR and CK 5/6, as proposed by P. Tang et al in his study.[8] This study aims to subtype breast carcinoma based on molecular classification using immunohistochemistry as surrogate markers and to correlate them with tumor morphology, histologic grade, pathological stage and clinical findings. We then correlated the molecular subtypes of breast carcinoma with 5-year survival in each subtype.

Materials and Methods

The present study is from a Multispecialty Government Hospital with attached 16 dispensaries spread across the city of Mumbai providing universal health care to 90,000 working as well as retired government employees and their dependents, living in Mumbai. The healthcare services are

provided under the Contributory Health Service Scheme and covers preventive, diagnostic and treatment for all included in the population. This includes breast cancer screening for all women to detect breast cancer cases at an early stage. All cases diagnosed as cancer undergo standard treatment protocols with regular follow up thereafter. Case records are archived in the Hospital information management system.

Case Selection

The present study included 158 consecutive surgical specimens diagnosed as invasive breast carcinoma between 2007 and 2017. The H&E stained sections were reviewed to study tumor morphology, grade and stage. Representative blocks of tumor were selected for immunohistochemistry. All cases were stained for ER, PR, HER2 and Ki67. Based on results of ER, PR, HER2, and Ki67, sections were further stained for CK 5/6 and EGFR. Interpretation was done using standard guidelines i.e., ASCO/CAP recommendations.[9] Criteria used for interpretation of IHCs are as follows:

I. ***ER/ PR Reporting****:* Allred scoring system was used. A total score of 3 and above were considered positive

II. ***Her2neu Reporting*****:** The ASCO/CAP guideline recommends that HER2 be defined as positive if 10% or more of tumor cells exhibit strong uniform membrane staining. Based on the CAP/ASCO guidelines and using a protocol similar to a pioneering study by P. Tang et al [8], we classified invasive breast carcinomas in the present study as HER2 positive if 10% or more of tumor cells exhibit strong uniform membrane staining.

III. ***Ki67 Reporting:*** Although Ki-67 LI is a useful biomarker for differentiating the luminal B subtype from the luminal A subtype of breast cancer, there is no established cutoff point. P. Tang [8] in his study, defined luminal A as ER+, HER2-, and Ki-67 of less than 14% with any PR, or Ki-67 of 14% to 19% with PR greater than 20%; and luminal B as ER+, HER2-, and Ki-67 of greater than 14% with any PR, or Ki-67 of 14% to 19% and PR less than 20%. We have used the same criteria for subtyping Luminal tumors.

IV. ***CK 5/6:*** As per various reports in literature cut offs for its positivity range from any positive cytoplasmic staining to 20% of tumor cells. Since no standard cut-off is described, we have used moderate to strong cytoplasmic positivity in more than 20% tumor cells as positive.

V. ***EGFR:*** Positivity was reported for cases having intermediate and strong staining, (dark brown linear membrane staining) in more than 10 percent tumor cells.

Tumors were classified based on ER, PR, HER2, CK-5, CK6, KI 67 and EGFR results in to 7 molecular subtypes as was done in study of Tang et al [8] [Table 1]

Data collection: Case files of the patients for information regarding clinical finding, family history, sonography, and mammography findings, were studied from HIMS system which stores complete patient records including follow-up. Each patient is identified by a unique identification number which remains the same for Universal health coverage and records are easily retrievable.

Data Collection Forms**:** Data including the medical history personal history and physical examination, sonography and mammography findings were entered into data collection forms directly. Data entered in Data collection forms, and the results of Slide review & IHC was entered into data sheet using Microsoft office 2007 for the study and for statistical Analysis.

Statistical Analysis: **D**ata was analyzed using Excel sheet and with the help of SPSS Software version 21. Quantitative data was presented with the help of Mean, Standard Deviation, and Median. Qualitative data was presented with the help of Frequency and Percentage table. Association among two or more subtypes of breast carcinoma and morphological types (qualitative data) was assessed with the help of Chi-Square test. The correlation among two or more Molecular subtypes of breast carcinoma and various clinicopathological parameters was compared by using Non-Parametric Test (Mann Whitney Test or Wilcoxon Sign Rank Test). Tumors thus classified were correlated with clinical findings like age, menstrual history, family history of breast cancer, morphological classification, grade, tumor size, presence of lymphovascular invasion, lymph node involvement, pathological stage and presence of metastases.

The five-year relative survival rate in breast carcinoma patients was studied by Kaplan Meir life table anaysis.

The study was approved by the Institutional scientific committee (ref no BARCHMEC/34/2016 and 09/08/17) and the Medical Ethics committee (Ref no: project no. BHMEC/DNB/06/2017 and 13/09/2017).

Result

Among the 158 cases in the present study, Luminal A subtype- 49 cases (31.0%) was found to be most common subtype, followed by Luminal B- 40 cases (25.3%), NCBC- 23 cases (14.6%), HER2 Enriched- 21 cases (13.3%) and

BLBC- 15 cases (9.5%). Cases Positive for both ER and HER2 were categorized as Luminal HER2, which was sub classified as Luminal HER2 PR+ - 7 cases (4.4%) and Luminal HER2 PR- -3 cases (1.9%) [Figures 1 -7].

Clinical and pathological findings of all 158 cases are shown in Table 1. There were 157 females, and one male. The mean age of presentation was 59.85 years. The youngest age of presentation was 29 years and there was total 7 cases less than 40 years. Of the 41-80 years age group, majority of the cases were Luminal A (33.5%), followed by Luminal B (25.8%) and triple- negative subtypes (NCBC -13.9% & BLBC-9.7% respectively). HER2+ cases account for 20.9% in this age group. Among the seven cases less than 40 years, three cases were classified as NCBC, two cases were Luminal B and one case each was of Luminal A and HER2 Enriched type.

Out of 157 IBC cases in females, 139 cases (88.5%) were in postmenopausal women. Of these, Luminal A subtype was 47 cases (33.8%) and Luminal B was 34 cases (24.4%). Among premenopausal women (18/157cases), most cases were Luminal B and NCBC i.e. 27.8% each and Luminal A subtype was only 11.1%. Family history of breast cancer was present in 19 cases (12%) out of 158.

135 cases (85.4%) were histologically diagnosed as IBC, NST, subtypes were Luminal A (31.8%) followed by Luminal B (25.9%) and HER2 positive (21.4%). Six out of seven cases of medullary carcinoma belonged to triple-negative subtype which were further grouped as NCBC (5 cases) and BLBC (1case). We observed that the well differentiated tumors like cribriform carcinoma (2 cases), papillary carcinoma (1 case), mucinous carcinoma (1 case) and signet ring cell carcinoma (1 case) were classified as Luminal A subtype. The two metaplastic carcinomas in our study had a triple- negative immunoprofile and were grouped one each under BLBC and NCBC subtype.

In the Luminal A subtype 61.2% were grade 2 tumors, followed by grade 1 (22.4%). However, most cases of Luminal B and HER2 positive subtypes were grade 3; 45.0% and 57.1% respectively. Of the triple negative category (BLBC & NCBC), 73% were Grade 3 tumors. This correlation was found to be statistically significant (p value < 0.001).

Maximum cases in the present study belonged to pathological stage T2 (110/158 cases i.e., 70%, followed by T1 (35/157 cases) i.e. 22.3%. Tumors belonging to all molecular subtypes had tumor size predominantly corresponding to T2 stage followed by T1.

87 out of 158 cases (55.4%) did not show lymph node metastasis (N0), 29.9% cases had metastases in up to three ipsilateral lymph nodes (N1), 9.5% cases had metastases in 4 to 9 ipsilateral axillary lymph nodes (N2) and 5.1% cases had metastases in more than 9 ipsilateral axillary lymph nodes (N3). 39.6% cases of Luminal A, 65% cases of Luminal B and 4.8% cases of HER2 enriched subtype presented with lymph node involvement. Our study shows significant correlation between Molecular subtype and nodal metastasis, ($p<0.015$).

25 cases out of 158 (15.8%), presented with distant metastases on follow-up. Among these, HER2 enriched subtype presented with larger proportion of cases with metastasis (28.5%), followed by BLBC (20.0%) and NCBC (17.4%). Common sites of distant metastases included brain (25.0%), liver (20.0%) and bone (15.0%). Metastasis to contralateral breast and lung was seen in 10% of cases. Cases having distant metastasis were categorized as stage group 4 based on TNM stage grouping (American Joint Committee on Cancer (AJCC) Cancer Staging Manual 7th ed). [9,10] These 25 cases of stage 4 tumor included 6 cases each of Luminal B subtype (24.0%) and HER2enriched subtype (24.0%), Luminal A -5 cases (20%), luminal HER2PR+ 1 case (20.0%), BLBC 3 cases (12.0%) and NCBC 4 cases (16.0%). No statistically significant correlation could be achieved.

Lymphovascular invasion wase present in 71 out of 158 cases (44.9%). It was observed that a greater proportion of tumors of HER2 + subtypes (45.1%) and triple-negative subtypes (39.4%) showed lymphovascular invasion. However, this correlation was not significant statistically (*p* value-0.89).

Most common treatment modality of our cases was MRM with chemotherapy (CT) or Hormonal therapy (39.2% cases). CT comprised of taxanes, Anthracyclines, platinum agents, 5- FU, Cytoxan agents given as intravenous two or three drug combination cycles and Hormonal therapy comprised of Tamoxifen/ Aromatase inhibitor. Breast Conservation Treatment (BCT) with CT was given in 25.3% cases. Radiotherapy in combination with Surgical treatment and chemotherapy was given in 23.4% of cases. 34.2% of triple negative subtype and 19.05% of HER2 (+) subtypes received radiotherapy.

Survival Analysis

In the present study, out of the 80 cases of Invasive breast carcinoma diagnosed between 2007 to 2013, 63 cases (78.7%) survived 5years with treatment and 17 were non survivors (21.3%). Most of the non-survivors were in HER2 Enriched subtype (33.3%), followed by Luminal B subtype (22.2%). This correlation is however not statistically

significant (*p* value- 0.32). The overall survival rate of IBC in our study is 79%. The survival rate for each subtype were as follows: Luminal A 88%; Luminal B subtype 67%; Luminal HER2PR+ 50% (only 2 cases); Luminal HER2PR- 0%; HER2Enriched 71%; BLBC 90%; NCBC 79% as seen in the Kaplan Meir survival graph [Figure 8].

Discussion

Among the 158 cases in the present study, Luminal A subtype- 49 cases (31.0%) was found to be most common subtype, followed by Luminal B- 40 cases (25.3%), NCBC-23 cases (14.6%), HER2 Enriched- 21 cases (13.3%) and BLBC 15 cases (9.5%). We observed that 34.1% belonged to age group of 61-70 years and mean age of presentation was 59.85yrs, which was comparable with other studies.[11,12] In the 61-70 years age group category, majority of the cases were Luminal A (37%) followed by Luminal B (22.2%). HER2+ cases accounted for 16.5% in this age group and triple negative subtypes were NCBC -14.8% and BLBC- 9.3%. These observations are concordant with other similar studies by Hadizadeh et al, Kumar et al and Fernandes et al.[12, 13, 14]

Table1: Molecular classification of invasive breast carcinoma using Immunohistochemical surrogates.

Luminal Breast cancer				HER2 positive BC			Triple Negative	
	Luminal A	Luminal B						
Molecular Subtypes	LA	Ki-67 ≥14%	PR <20%	Luminal HER2 PR+	Luminal HER2 PR-	HER2 Enriched	BLBC (Basal like)	NCBC (Non classifiable)
ER	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
PR	+ve	>20	<20	+ve	-ve	-ve	-ve	-ve
HER2	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve
Ki-67	<14	≥14	+/-	+/-	+/-	+/-	+/-	+/-
CK5	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
EGFR	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve

Table 2: Distribution of Cases of Invasive breast carcinoma.

variables	All cases N=158	Luminal A N=49 100%	Luminal B N=40	Luminal Her2PR+ N=7	Luminal Her2PR- N=3	Her2enriched N=21	Basal like N=15	Unclassified (NCBC) N=23	P value
Frequency	158	49(31.0)	40(25.3)	7(4.4)	3(1.9)	21(13.3)	15(9.5)	23(14.6)	**<0.001**
Mean Age	59.85	61.3	60.6	58.8	55	58.2	58.9	58.3	0.7716
Menstrual history	Post -Menopausal	47(95.9)	34(85.0)	7(100)	3(100)	19(90.5)	11(73.3)	18(78.3)	0.07865
	Pre-Menopausal	2(4.1)	5(15.0)	0(0.0)	0(0.0)	2(9.5)	4(26.7)	5(21.7)	
Grade	1(18)	11(22.4)	6(15.0)	0(0.0)	0(0.0)	0(0.0)	1(6.67)	0(0.0)	**<0.001**
	2(70)	30(61.2)	16(40.0)	5(71.4)	1(33.3)	9(42.8)	3(20.0)	6(26.1)	
	3(70)	8(16.3)	18(45.0)	2(28.6)	2(66.7)	12(57.1)	11(73.3)	17(73.9)	
Tumor size	T1	15(31.2)	9(22.5)	1(14.3)	1(33.3)	3(14.3)	3(20.0)	3(13.0)	0.5936
	T2	30(62.5)	29(72.5)	6(85.7)	2(66.7)	15(71.4)	11(73.3)	17(73.9)	
	T3	3(6.25)	2(5.0)	0(0.0)	0(0.0)	2(9.5)	1(6.7)	3(13.0)	
	T4	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.8)	0(0.0)	0(0.0)	
Nodal metastases	N0	29(60.4)	14(35.0)	5(71.4)	1(33.3)	12(57.1)	12(80.0)	14(60.8)	**<0.01**
	N1	14(29.2)	21(52.5)	1(14.3)	1(33.3)	2(9.5)	3(20.0)	5(21.7)	
	N2	4(8.3)	4(10.0)	0(0.0)	0(0.0)	4(19.0)	0(0.0)	3(13.0)	
	N3	1(2.1)	1(2.5)	1(14.3)	1(33.3)	3(14.3)	0(0.0)	1(4.3)	
Distant metastases on follow-up	25(15.8)	5(10.2)	6(15.0)	1(14.2)	0(0.0)	6(28.5)	3(20.0)	4(17.3)	0.486
TNM stage at diagnosis	1A	12(25.0)	5(12.5)	1(14.3)	0(0.0)	2(9.5)	2(13.3)	2(8.7)	**0.046**
	2A	18(37.5)	13(32.5)	4(57.1)	1(33.3)	10(47.6)	10(66.7)	13(56.5)	
	2B	13(27.1)	15(37.5)	1(14.3)	1(33.3)	2(9.5)	3(20.0)	2(8.7)	
	3A	3(6.3)	6(15.0)	0(0.0)	0(0.0)	4(19.0)	0(0.0)	5(21.7)	
	3B	1(2.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	
	3C	1(2.1)	1(2.5)	1(14.2)	1(33.3)	3(14.3)	0(0.0)	1(4.3)	

variables	All cases N=158	Luminal A N=49 100%	Luminal B N=40	Luminal Her2PR+ N=7	Luminal Her2PR- N=3	Her2enriched N=21	Basal like N=15	Unclassified (NCBC) N=23	P value
LVI +	71(44.9)	22(44.8)	20(50.0)	4(57.1)	2(66.6)	8(38.1)	6(40.0)	9(39.1)	0.8936
Survival 5yrs (2007-2013)	Survivors (63)	25(89.2)	6(66.6)	1(50.0)	0(0.0)	10(66.7)	9(90.0)	12(80.0)	0.3263
	Nonsurvivors (17)	3(10.7)	3(33.3)	1(50.0)	1(100)	5(33.3)	1(10.0)	3(20.0)	
	Total (80)	**28(35.0)**	**9(11.2)**	**2(2.5)**	**1(1.25)**	**15(18.7)**	**10(1.5)**	**15(18.7)**	

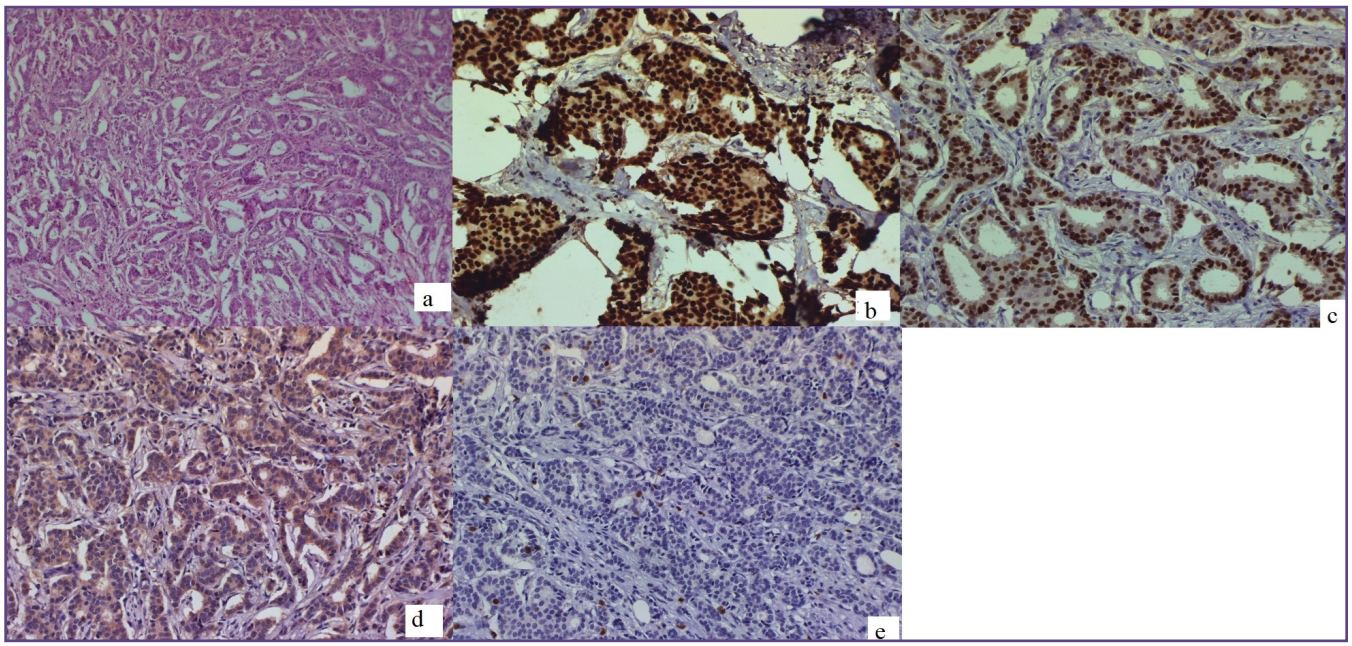

Fig. 1: Luminal A subtype: (a) IBC- NST, H & E (x100); (b) Intense nuclear ER Positivity (x200)); (c) Moderate nuclear PR positivity (x200); (d) Negative HER2 (x200); (e) Ki67 nuclear positivity in < 1% cells(x200).

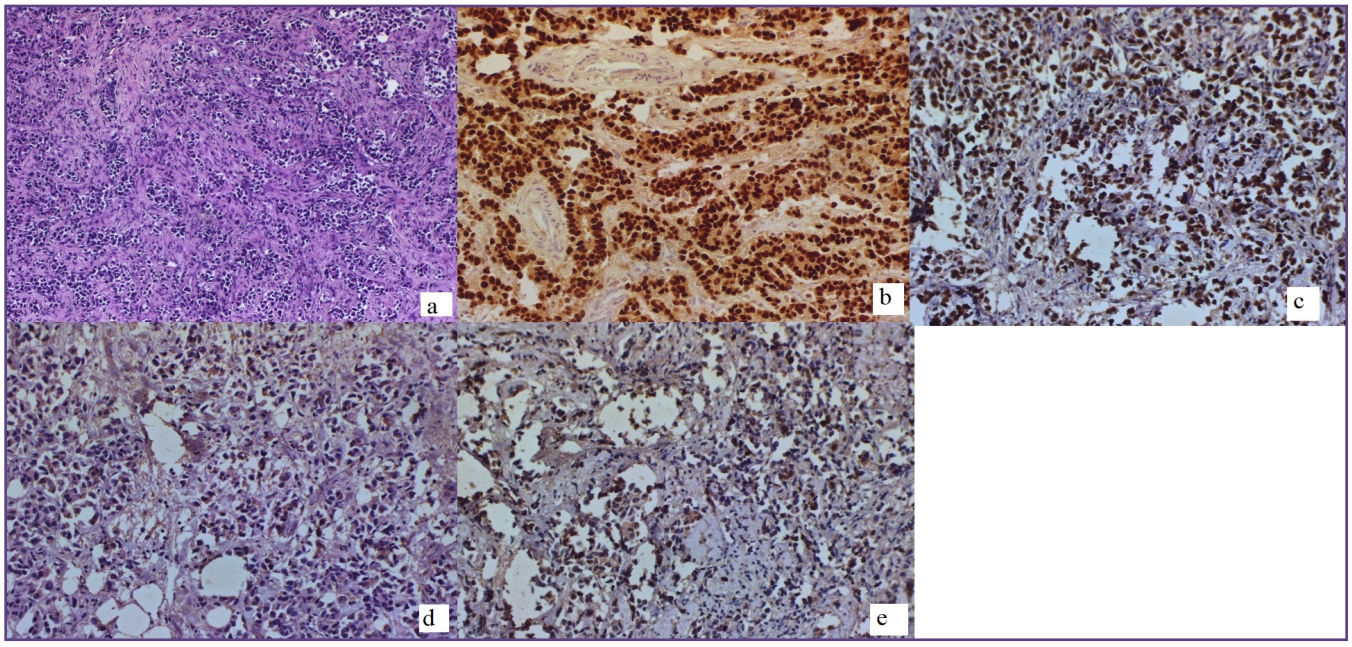

Fig. 2: Luminal B subtype: (a) IBC- NST, H & E (x100); (b) Intense nuclear ER Positivity (x200)); (c) Moderate nuclear PR positivity (x200); (d) Negative HER2 (x200); (e) Ki67 nuclear positivity in > 60% cells(x200).

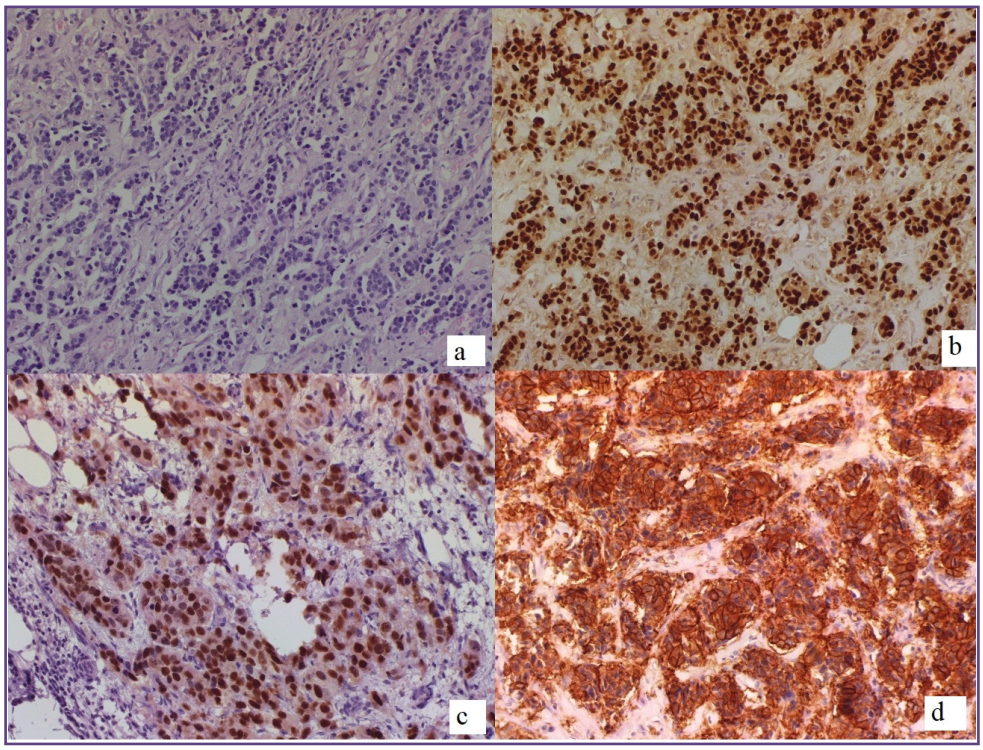

Fig. 3: Luminal HER2 PR (+) Subtype: (a) IBC- NST, H & E (x100); (b) Intense nuclear ER positivity (x200)); (c) Moderate nuclear PR positivity (x200); (d) HER2 membranous positivity > 10% cells(x200).

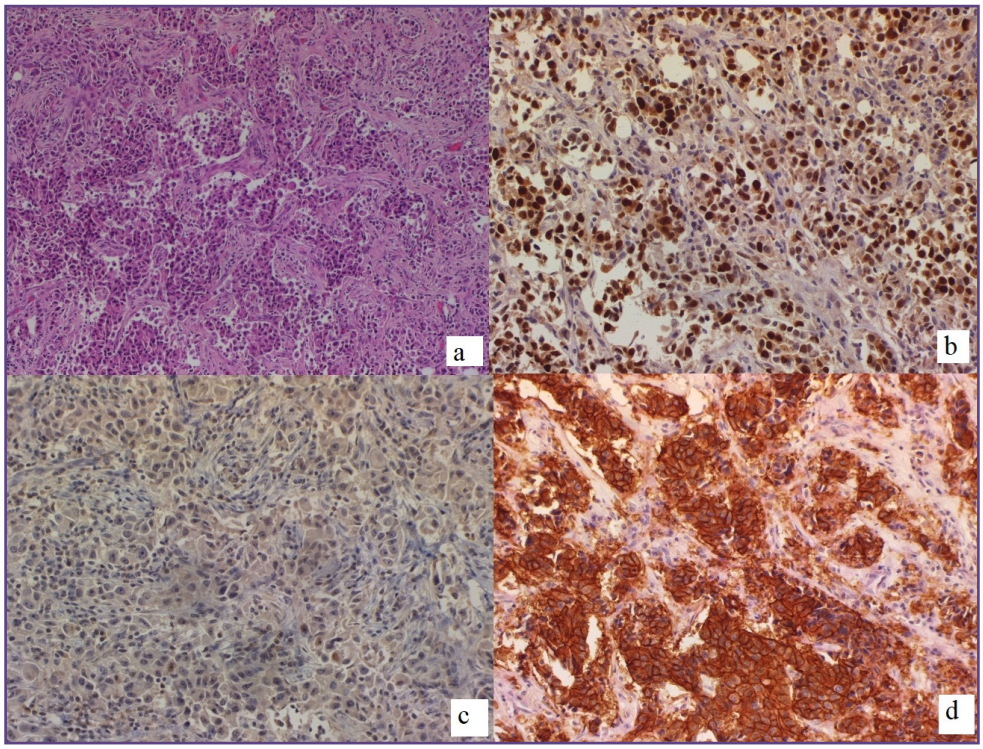

Fig. 4: Luminal HER2 PR (-) Subtype: (a) IBC- NST, H & E (x100); (b) Moderate nuclear ER positivity (x200)); (c) Negative PR (x200); (d) HER2 membranous positivity > 30% cells(x200).

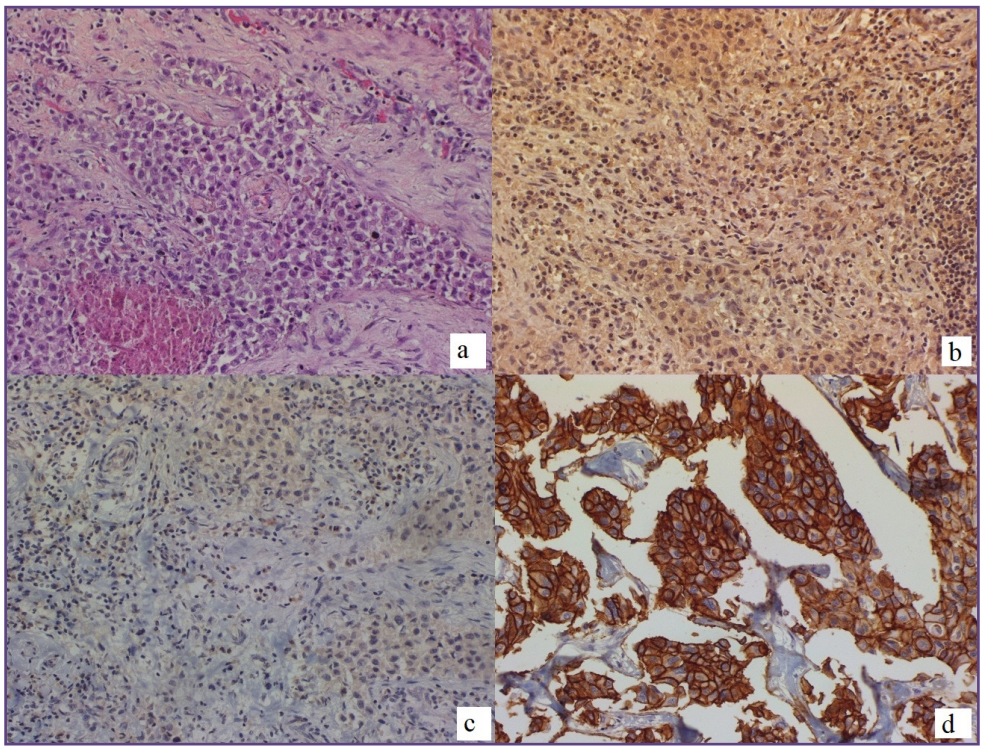

Fig. 5: HER2 Enriched Subtype: (a) IBC- NST, H & E (x100); (b) Negative ER (x200)); (c) Negative PR (x200); (d) HER2 membranous positivity > 30% cells(x200).

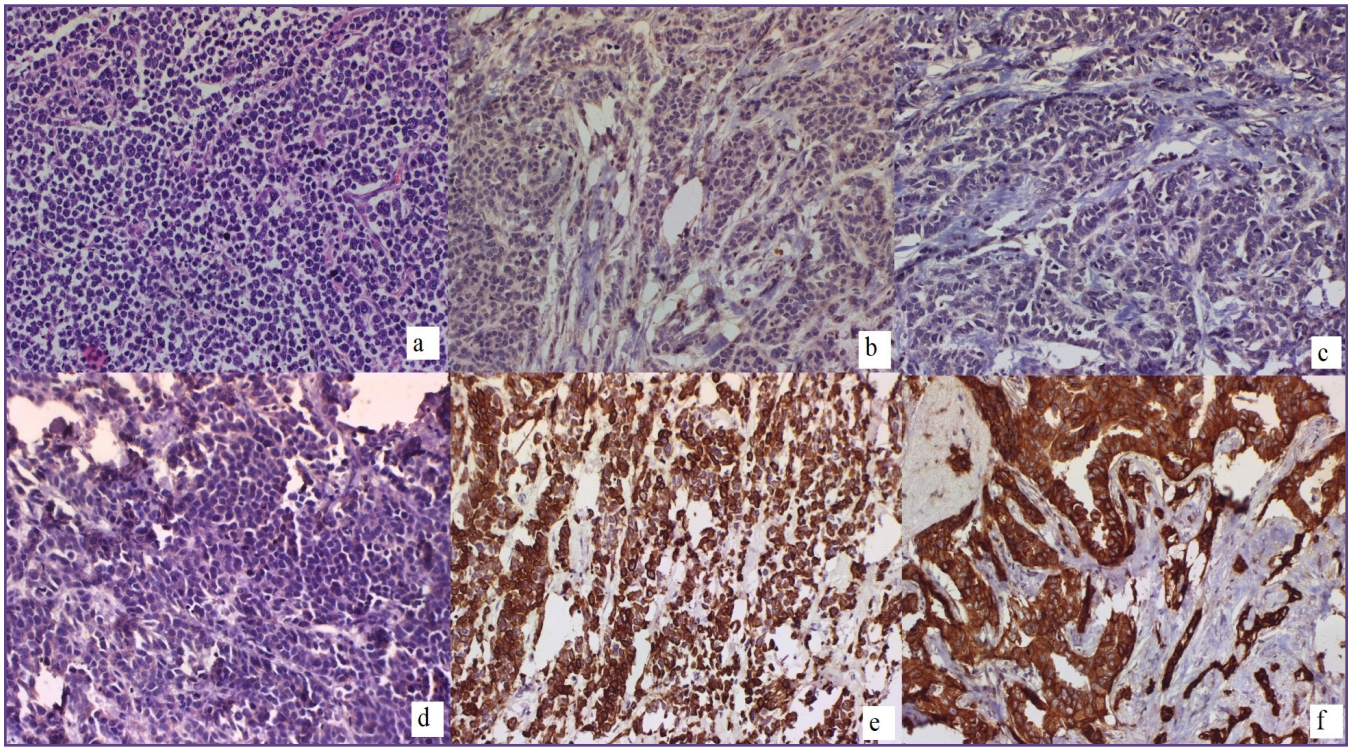

Fig. 6: Basal Like subtype: (a) IBC- NST, H & E (x100); (b) Negative ER (x200); (c) Negative PR (x200); (d) Negative HER2 (x200); (e) Cytoplasmic CK 5/6 positivity in cells(x200); (f) Membranous EGFR positivity in cells (x200)

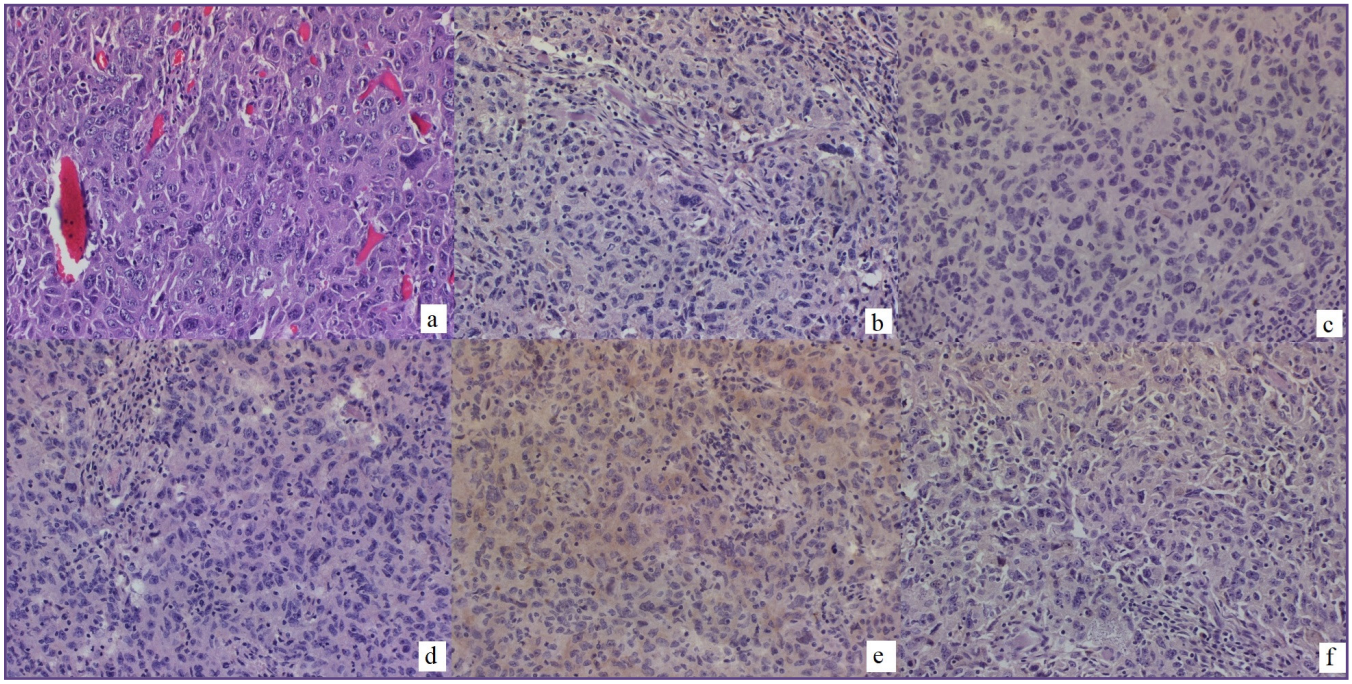

Fig. 7: Non-classifiable breast carcinoma: (a) IBC- NST, H & E (x100); (b) Negative ER (x200)); (c) Negative PR (x200); (d) Negative HER (x200); (e) Negative CK 5/6 (x200); (f) Negative EGFR(x200).

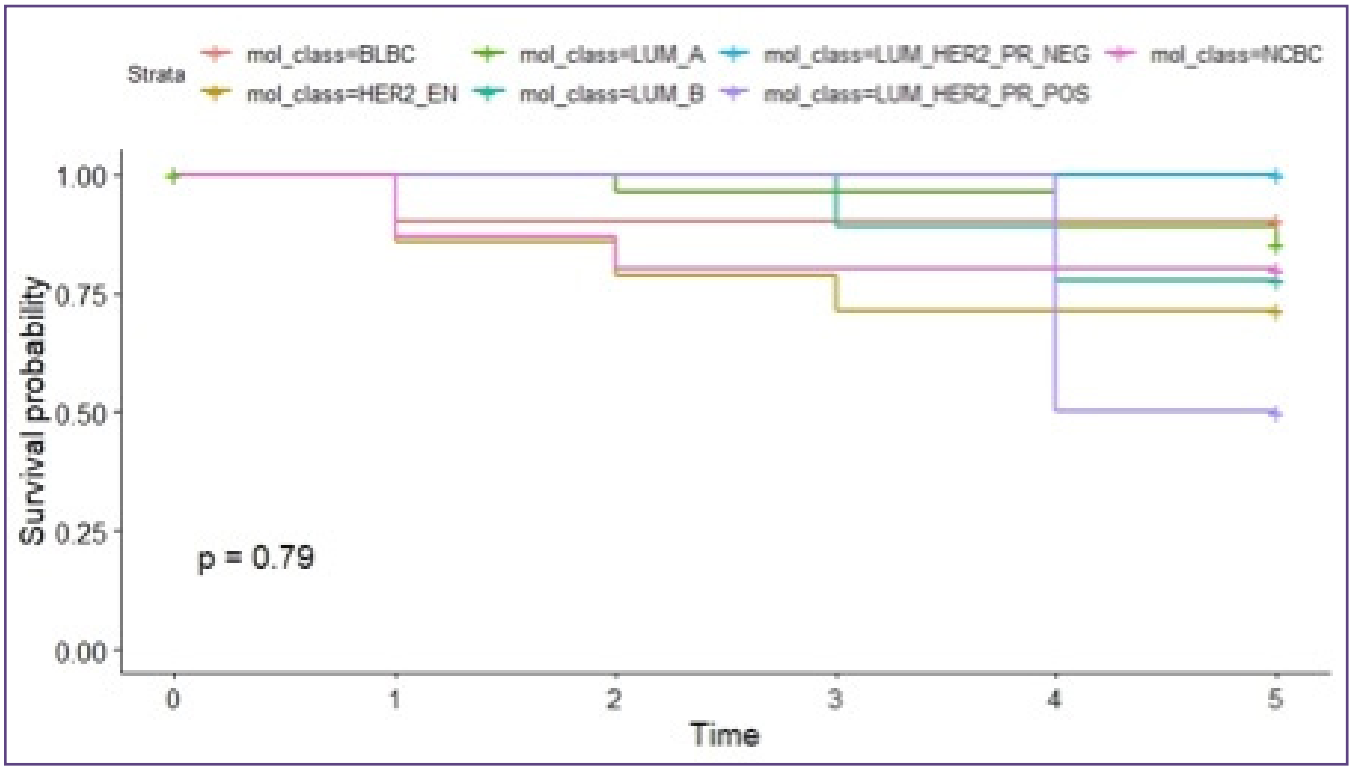

Fig. 8: Kaplan Meir survival graph depicting five- year survival amongst molecular subtypes of invasive breast carcinoma.

We also observed that amongst the age group of less than 40 years, four out of 7 cases (57.1%) were HER2 positive and Triple marker negative (ER/ PR/ HER2 negative).

Molecular classification and histological type

In our study, Luminal A molecular subtype (49 cases) comprised of cases of IBC, NST (43 cases), Invasive cribriform carcinoma (2 cases), and one each case of invasive papillary carcinoma, invasive lobular carcinoma, mucinous carcinoma and signet ring cell carcinoma. Luminal B cases (40 cases) included 34 cases of IBC, NST, 4 cases of invasive lobular carcinoma and one case of invasive papillary carcinoma. All the 10 cases classified as Luminal HER2 were diagnosed as IBC- NST. HER2 enriched subtype comprised of 19 cases of IBC, NST and one case of IBC with medullary features. The Triple Negative Breast Cancers (TNBC) comprised 28 cases of IBC- NST, 7 cases of IBC with medullary features, and 2 cases each of invasive lobular carcinoma and metaplastic carcinoma.

Our findings are comparable with other studies in literature. Vuong D et al, in his study has reported that morphologically, most Luminal A tumors are well differentiated carcinomas of no special type, tubular carcinomas, classical lobular carcinomas, mucinous carcinomas, and neuroendocrine carcinomas.[15] Luminal B group of tumors are less well differentiated and consists mostly of IBC of NST. Other studies, like Calderella et al and Mahajan et al have reported medullary carcinoma as being mostly subtyped as triple negative breast cancer, having younger age of presentation and adverse pathological characteristics, [16, 17] which is much similar to our findings in six out of seven cases (85.7%) of medullary carcinoma.

Molecular subtype and grade of tumor

In the present study, 61.2% of Luminal A tumors were grade 2 tumors, whereas, most cases of Luminal B, and HER2 positive tumors were grade 3 tumors i.e., 45.0% and 57.1% respectively. 40% cases of Luminal B tumors were grade 2. This correlation was found to be statistically significant (p value< 0.001). This finding is in agreement with other studies by De Laurentiis et al and Parise et al where Luminal A tumors were found to be better differentiated and mainly grade 2 tumors and Luminal B found to be grade 2 or 3 tumors. [18,6]

Similar to the present study, Alqaisi et al further classified luminal HER2 subtype into two phenotypes based on PR expression: ER+/PR+/HER2+ (triple-positive cancer) and ER+/PR-/HER2+, each having distinct clinical properties as younger age of presentation and aggressive behavior and stronger estrogen dependence in former.[19] We had 7 cases of Luminal HER2 PR (+), of which 5 cases (71.4%) were grade 2 and two cases were grade 3. Out of 3 cases of Luminal HER2 PR (-), two cases were grade 3 (66.6%). A larger series of cases of these categories needs to be studied, to establish clinical significance of this finding and to study the necessity to separate the two categories.

Maximum cases in the category of HER2 enriched, BLBC and NCBC belonged to grade 3 tumors, i.e. 57.1%, 73.3% and 73.9 % respectively, which is statistically significant (p value < 0.001) and comparable with other studies in literature.[6,8] When molecular subtypes were correlated with grade, our findings are similar to study by Parise et al which showed that majority of Luminal cases are in grade 1 and 2 and as grade advances there is relative increase in proportion of HER2 positive and triple negative subtypes.[6]

Tumor size and Molecular subtypes

Similar studies in literature including those reported by Tiwari et al, Kumar et al, Calderella et al and Zaha et al have reported that T1 and T2 tumors were mainly of Luminal subtypes and a greater number of T3 tumors were subtyped as HER2 enriched and Triple negative subtypes. [11,13,16,20]

In the present study, 92.3% of all cases presented with tumor size less than 5 cm. Maximum cases in each subtype were diagnosed with tumor size less than 5 cm (T2). The reason for diagnosing most cases of breast cancers as T2 in the population studied may be due to comprehensive screening of all women in our study population leading to early diagnosis of most cases.

Molecular subtype and Nodal involvement

Positive nodes are one of the independent prognostic markers in the assessment of IBC. Out of 158 cases evaluated for nodal involvement, 87 cases did not show lymph node metastasis N0 (55.4%). Lymph node metastasis was observed in 39.6% and 65% cases of Luminal A and Luminal B subtypes respectively. Luminal HER2 subtype, HER2enriched subtype, BLBC and NCBC cases presented with 40%, 42.8%, 20% and 34.8% lymph node involvement respectively.

In the study by Zaha et al, 65.6% luminal B tumors were found to have nodal involvement followed by luminal A (58.6%) which is consistent with our study.[20] Similar findings were observed in reports by Tiwari et al and Kumar et al. [11,13]

Molecular subtype and lymphovascular invasion

In present study, 71 out of 158 cases i.e., 44.9% cases showed lymphovascular invasion. These include 30.9% cases of luminal A subtype and 28.1% cases of Luminal B

subtype. However, in other reports, that studied molecular subtypes in correlation with presence of lymphovascular invasion, like Kumar et al and Liao et al, lymphovascular invasion was predominantly reported in Luminal B subtype, followed by Luminal A and HER2 + cases. [13, 21]

Molecular subtype and distant metastasis

In present study, 25 cases among 158 cases had distant metastasis (15.8%) on follow-up. HER2 enriched molecular subtype presented with metastasis in 28.5% cases followed by BLBC (20.0%), NCBC (17.4%), luminal B (15.0%), LumHER2pos 14.3% (*p* value 0.48). The findings in our study are comparable with study by Yue Gong et al and Gerratana L et al, where majority of cases with metastases belonged to HER2 positive subtype. [22,25]

5yr survival rate in cases between 2007 to 2013 post therapy

Out of 80 cases diagnosed between 2007 to 2013, 63(78.7%) survived 5years with treatment. Of these 80 cases, majority belonged to Luminal A subtype (35%) and this group had the best survival of 88%. According to Zaha et al the 3yr survival and 5yr survival is highest in Luminal A and lowest survival is seen in basal subtype.[20] Gong et al in their study showed that HER2-enriched subtypes had significantly lower median survival rates compared with that of the luminal A and luminal B sub-types.[22]

Conclusion

IBC can be classified into molecular subtypes using immunohistochemistry as surrogates. Luminal A was the commonest followed by Luminal B, NCBC and HER2 Enriched subtype. The study highlighted that younger age group, less than 40 years was associated with higher proportion of HER2 positive and Triple Negative subtypes. While Luminal A subtypes were mostly IBC- NST and other well differentiated tumors, mostly grade 2; Luminal B were mostly IBC- NST cases of grade 3, with higher proportion of lymph nodal involvement, which are independent prognostic factors indicating poorer prognosis.

Maximum cases of HER2 enriched, BLBC and NCBC belonged to grade 3 type of tumors (p value< 0.001), which was also associated with higher proportion of cases with distant metastasis on follow-up, indicating poor prognosis and need for close follow-up. Hence in the present study we could conclude that molecular subtyping of IBC should be done in all cases not only for planning management but also for prognostication.

The overall survival rate of IBC in our study is 79%. However, a larger sample with a greater number of cases in each subtype needs to be studied for evaluation of survival in each subtype.

Abbrevations

IBC – Invasive Breast Carcinoma

IHC – Immunohistochemistry

ER – Estrogen Receptor

PR- Progesterone Receptor

HER 2 – Human Epidermal Receptor 2 neu

EGFR- Epidermal Growth Factor Receptor

CK- Cytokeratin

BLBC – Basal Like Breast Carcinoma

NCBC – Non-Classifiable Breast Carcinoma

Acknowledgements

Mrs Sulabha Pednekar, Smt Sanjana Sherkhane and Mr Kishore Kudikala, histopathology technologists provided technical help.

Funding

Nil

Competing Interests

Nil

Reference

1. The GLOBOCAN 2018 database, IARC Global Cancer Observatory, Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018, PRESS RELEASE N° 263, Geneva, Switzerland, 12 September 2018.
2. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013.
3. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer 2013; 132:1133-45.
4. National Cancer Registry Programme. Consolidated report of the population-based cancer registries 1990-1996. New Delhi: Indian Council of Medical Research; 2001.
5. van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002; 47:1999–2009.
6. Parise CA, Caggiano V. Breast Cancer Survival Defined by the ER/PR/HER2 Subtypes and a Surrogate Classification according to Tumor Grade and Immunohistochemical Biomarkers. J Cancer Epidemiol 2014; 2014:469251.
7. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn HJ; Panel members. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert

Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013; 24:2206-23.

8. Tang P, Tse GM. Immunohistochemical Surrogates for Molecular Classification of Breast Carcinoma: A 2015 Update. Arch Pathol Lab Med 2016; 140:806-14.
9. American Joint Committee on Cancer (AJCC) Cancer Staging Manual 7th ed. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti III H. eds. New York: Springer. 2009.
10. International Union against Cancer (UICC): TNM classification of malignant tumors 7th ed. Sobin LH, Gospodarowicz MK, Wittekind Ch. Eds. Wiley-Blackwell. Oxford. 2009.
11. Tiwari S, Malik R, Trichal VK, Nigam RK, Rai A, Balani S et al. Breast Cancer: Correlation of Molecular Classification with Clinicohistopathology. Sch. J. App. Med. Sci 2015; 3:1018-26.
12. Hadizadeh, M.; Arani, H. Z.; Olya, M. Expression of Breast Cancer Subtypes Based on the Most Important Biomarkers: Comparison of Clinicopathological Factors and Survival. Iran Red Crescent Med J 2018;20: e57931.
13. Kumar N, Patni P, Agarwal A, Khan MA, Parashar N. Prevalence of molecular subtypes of invasive breast cancer: A retrospective study. Med J Armed Forces India 2015; 71:254-8.
14. Fernandes RC, Bevilacqua JL, Soares IC, Siqueira SA, Pires L, Hegg R, Carvalho FM. Coordinated expression of ER, PR and HER2 define different prognostic subtypes among poorly differentiated breast carcinomas. Histopathology 2009; 55:346-52.
15. Vuong D, Simpson PT, Green B, Cummings MC, Lakhani SR. Molecular classification of breast cancer. Virchows Arch 2014; 465:1-14.
16. Calderella A, Buzzoni C, Crocetti E, Bianchi S, Vezzosi V, Apicella P et al. Invasive breast cancer: a significant correlation between histological types and molecular subgroups. J Cancer Res Clin Oncol 2013:139:617–23.
17. Mahajan S, Singh A, Joneja A, Kapoor S. Molecular Evaluation of Subtypes of Breast Cancer by Immunohistochemical expression of Estrogen Receptor (ER), Progesterone Receptor (PR), HER2neu, Cytokeratin 5/6 and Ki 67. International Journal of Science and Research (IJSR) 2016; 5:184-8.
18. De Laurentiis M, Arpino G, Massarelli E, Ruggiero A, Carlomagno C, Ciardiello F, Tortora G, D'Agostino D, Caputo F, Cancello G, Montagna E, Malorni L, Zinno L, Lauria R, Bianco AR, De Placido S. A meta-analysis on the interaction between HER-2 expression and response to endocrine treatment in advanced breast cancer. Clin Cancer Res. 2005; 11:4741-8
19. Alqaisi A, Chen L, Romond E, Chambers M, Stevens M, Pasley G, Awasthi M, Massarweh S. Impact of estrogen receptor (ER) and human epidermal growth factor receptor-2 (HER2) co-expression on breast cancer disease characteristics: implications for tumor biology and research. Breast Cancer Res Treat 2014;148(2):437-44.
20. Zaha DC, Lazăr E, Lăzureanu C. Clinicopathologic features and five years survival analysis in molecular subtypes of breast cancer. Romanian Journal of Morphology and Embryology = Revue Roumaine de Morphologie et Embryologie 2010 ;51(1):85-9.
21. Liao GS, Hsu HM, Chu CH, Hong ZJ, Fu C, Chou YC et al. Prognostic role of lymphovascular invasion and lymph node status among breast cancer subtypes. J Med Sci 2018; 38: 54- 61.
22. Gong Y, Liu YR, Ji P, Hu X, Shao ZM. Impact of molecular subtypes on metastatic breast cancer patients: a SEER population-based study. Sci Rep 2017; 7:45411.
23. Mao JH, Diest PJV, Perez-Losada J, Snijders AM. Revisiting the impact of age and molecular subtype on overall survival after radiotherapy in breast cancer patients. Sci Rep 2017; 7:12587.
24. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 2009; 101:736-50.
25. Gerratana L, Fanotto V, Bonotto M, Bolzonello S, Minisini AM, Fasola G, Puglisi F. Pattern of metastasis and outcome in patients with breast cancer. Clin Exp Metastasis 2015; 32:125-33.

***Corresponding author:**
Dr. Susan Cherian, Head Pathology department, BARC Hospital, Anushaktinagar, Mumbai-400094, Maharashtra, India
Phone: +91 9833884370
Email: cheriansusan@yahoo.com

Financial or other Competing Interests: None.

Date of Submission : 11/05/2021
Date of Final Revision : 19/08/2021
Date of Acceptance : 06/09/2021
Date of Publication : 30/09/2021