Original Article

St. Gallen's molecular subtypes in primary breast carcinoma in Indian population

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ABSTRACT

Aim: Immunohistochemical (IHC) markers have been used as surrogates for DNA-microarray in subtyping breast cancer, which lead to new biological insights and eventually to better-targeted therapies. Our study aimed at studying the distribution of the St. Gallen's molecular subtypes in our population and to evaluate their association with traditional prognostic features. **Materials and Methods:** Seventy-five cases of primary breast cancer undergoing radical or modified radical mastectomy were classified into five subtypes based on their IHC profile using estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (Her2)/neu, and Ki-67 as per St. Gallen's guidelines. IHC subtypes were correlated with various clinicopathologic prognostic parameters including age, tumor size, tumor type, axillary lymph node status, and histologic tumor grade. **Results:** The proportion of each subtype in our population was: luminal A 28%, luminal B Her2− 18.7%, luminal B Her2+ 9.3%, Her2/neu+ 17.3%, basal cell-like (BCL) 26.7%. The majority of luminal A cases were well differentiated (Grade I) whereas luminal B Her2− was mostly moderately differentiated (Grade II) and luminal B Her2+ constituted mainly well differentiated and moderately differentiated tumors. Both subtypes of luminal B showed lymph node metastasis in majority of the cases. Her2/neu+ and BCL subtype were high-grade tumors comprising mainly of moderately differentiated and poorly differentiated tumors. The majority of luminal A cases were negative for Ki-67 whereas all the luminal B Her2− tumors expressed Ki-67, 50% being highly positive. BCL subtypes revealed highest proliferation index with 80% of the cases being high positive Ki-67. A statistically significant difference of modified Bloom–Richardson grade and Ki-67 distribution ($\chi^2 = 42.974$; $P < 0.001$) between various IHC subtypes was observed.

Key words: Breast carcinoma, immunohistochemical subtypes, molecular classification, St. Gallen's

INTRODUCTION

Breast carcinoma is the most common malignant tumor and leading cause of carcinoma death in women, with more than 1,000,000 cases occurring worldwide annually.[1] India is facing a potential breast cancer epidemic over the next decade as women adopt Western lifestyle by marrying and bearing children later in life.[2]

In the past two decades, treatment of breast cancer has undergone dramatic change and a much wider range of therapeutic options are now available. As the range of options for treatment widens, it becomes increasingly important that the clinician is provided with accurate prognostic information on which to base the therapeutic decision.[3] However, the established clinical and pathologic metrics are no longer sufficient as a basis for the increasingly complex treatment decisions that are required.[4] Therefore, we need to discover new and more accurate prognostic predictors.

The classification of breast cancers into molecular subgroups on the basis of gene expression patterns in tumor tissue is often regarded as the gold standard.[5] It is useful for clinical management and is superior to the

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Access this article online

Quick Response Code:

Website: www.ccij-online.org

DOI: 10.4103/2278-0513.197860

Cite this article as: Batra A, Marwah N, Marwah S, Gupta S, Dharembra D, Sen R. St. Gallen's molecular subtypes in primary breast carcinoma in Indian population. Clin Cancer Investig J 2016;5:416-23.

WHO classification in short-term prognostic value. Breast cancers have been categorized into at least five main groups which differ markedly in terms of distinct races/ethnicities, risk factors distribution, prognosis, therapeutic treatment responsiveness, clinical outcomes, and both overall survival and relapse-free survival.[6] However, use of gene expression profiling in either the clinical or the research setting remains limited due to the expense and technical difficulties encountered.[5]

Immunohistochemical (IHC) surrogates for molecular classification for breast cancer are widely used. Various authors have variably used estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (Her2)/neu, cytokeratin (CK), and Ki-67 to categorize primary breast carcinoma into various IHC subtypes: luminal A (ER and/or PR+, Her2/neu−); luminal B (ER and/or PR+, Her2/neu+); Her2+ (ER−, PR−, Her2/neu+); and BASAL cell-like (BCL)/triple negative (ER−, PR−, Her2/neu−).[6-8] Ki-67 (a proliferative marker) has also been used to categorize luminal cancers into two subgroups: one with a low proliferative index and the other showing high Ki-67. The cutoff value to discriminate between low and high proliferative index groups is not consistent, i.e., 14%[9] or 20%[10] depending on different studies.

IHC classification although a feasible and recent tool in prognostication, has not been universally standardized yet. Different authors have used a different set of immunomarkers and subcategorized the cases into various subtypes. The IHC surrogate of molecular classification proposed by St. Gallen International Expert Consensus on the primary therapy of breast cancer can be used as a universal standard to bring out universal uniformity in IHC subtyping of breast cancer. Only a few studies on IHC characterization of breast cancer have been carried out in India,[11,12] of which only one is from North India, and none of them have used St. Gallen's molecular classification.[13] The aim of our study was to do IHC categorization of primary breast carcinoma according to St. Gallen's recent guidelines in North Indian population.

MATERIALS AND METHODS

The study was conducted on radical or modified radical mastectomy specimen of 75 consecutive cases of primary breast carcinoma. Histopathological diagnosis along with all the histologic parameters including grading by modified Bloom–Richardson (MBR) system and Nottingham prognostic index (NPI) was assessed.

Tissue sections (3–4 μ) from formalin-fixed, paraffin-embedded block of each case were taken on poly-L-lysine coated slides. After deparaffinization in xylene and rehydration through serial concentrations of alcohol, antigen retrieval was done in Tris ethylenediaminetetraacetic acid in pressure cooker. Endogenous peroxidase activity was blocked using peroxidase block (H_2O_2; BioGenex) for 20 min, followed by protein block (goat serum; BioGenex) for 15 min. Thereafter, the slides were incubated with one of the following primary antibodies: antihuman ER, PR, Her2/neu, and Ki-67 (rabbit monoclonal antibodies; Dako). Following treatment with secondary antibody (goat anti-rabbit antibodies; BioGenex) for 30 min and 3, 3′-diaminobenzidine tetrahydrochloride chromogen for 5 min, the slides were counterstained with Harris hematoxylin. Appropriate positive and negative controls were run with each IHC batch.

ER and PR staining was assessed using Quick score based on an assessment of proportion and intensity.[3] A score of ≥3 was taken as positive. Her2/neu positivity was determined by intense membrane staining of >30% of the tumor cells. Ki-67 labeling index (LI) was estimated after counting a minimum of 1000 cells in 10 high-power field and was expressed as a percentage of positive nuclei. Ki-67 LI of >5% and >20% was considered as positive and high positive, respectively.[10,14,15]

All the cases were subdivided into five subtypes based on their IHC profile as per St. Gallen's guidelines.[13]

The results obtained were subjected to statistical analysis. Mean and standard deviations were calculated. When the data were categorized, a Chi-square test was used to assess the association between these parameters. $P < 0.05$ and $P < 0.01$ were considered significant (S) and highly significant (HS), respectively. Mean age and mean tumor size were compared within different IHC subtypes using analysis of variance (ANOVA). *Post hoc* test was done to assess which IHC subtypes were different from others.

RESULTS

A total of 75 cases of breast carcinoma undergoing radical or modified radical mastectomy were included in the study. The clinicopathologic parameters are as shown in Table 1.

All the cases in our study were classified into five IHC subtypes based on ER, PR, Her2/neu, and Ki-67 expression. The IHC classification used in the study was as follows: luminal A: ER+ and/or PR+, Her2−, Ki-67 low (<14%) [Figures 1 and 2]; luminal B subdivided into two (a) luminal B Her2-negative: ER+ and/or PR+, Her2−, Ki-67 high (>14%) [Figures 3 and 4] and (b) luminal B Her2-positive: ER+ and/or PR+, Her2+, any Ki-67 [Figures 5 and 6]; ErbB2

Table 1: Clinicopathologic parameters of the study

Clinicopathologic characteristics	Number (Percentage)
Total number of cases	75
Age (years)	
Range	30-78
Mean	49.1±9.8
Menopausal status, *n* (%)	
Premenopausal	37 (49.3)
Postmenopausal	38 (50.7)
Histology, *n* (%)	
Ductal	66 (88)
Medullary	4 (5.4)
Lobular	2 (2.7)
Mucinous	1 (1.3)
Secretory	1 (1.3)
Metaplastic	1 (1.3)
Tumor size (cm), *n* (%)	
<2	16 (21.3)
2-4.9	51 (68)
≥5	8 (10.7)
MBR, n (%)	
Grade 1	23 (30.7)
Grade 2	39 (52)
Grade 3	13 (17.3)
Lymph node status, *n* (%)	
Negative	25 (33.3)
Positive	50 (66.7)
NPI, n (%)	
Good	12 (16)
Moderate	43 (57.3)
Poor	20 (26.7)
Ki-67, *n* (%)	
Negative (<5%)	20 (26.7)
Positive (>5%)	55 (73.3)
ER/PR, *n* (%)	
ER+/PR+	31 (41.3)
ER+/PR−	8 (10.7)
ER−/PR+	3 (4)
ER−/PR−	33 (44)
Her2, *n* (%)	
Positive	20 (26.7)
Negative	55 (73.3)

Her2: Human epidermal growth factor receptor 2, ER: Estrogen receptor, PR: Progesterone receptor, MBR: Modified Bloom–Richardson

overexpression or Her2/neu-positive (nonluminal): ER−, PR−, Her2+ [Figures 7 and 8] and BCL or triple negative: ER−, PR−, Her2− [Figures 9 and 10].[13]

Luminal A subtype constituted the maximum number of cases (28%) followed by basal-like (26.7%). Luminal B Her2− and Her2/neu+ subtype constituted 18.7% and 17.3% cases. Luminal B Her2+ was least common (9.3%). Invasive ductal carcinoma, not otherwise specified (NOS) constituted the predominant histological type in all IHC subtypes. Two of the four cases of medullary carcinoma belonged to Her2/neu+ group. One case of metaplastic carcinoma included was triple negative.

Luminal A and luminal B Her2− subtypes were more common in >50 years of age group, whereas luminal B Her2−, Her2/neu+, and basal-like were seen more in age < 50 years. Luminal B Her2−, Her2/neu+, and basal-like

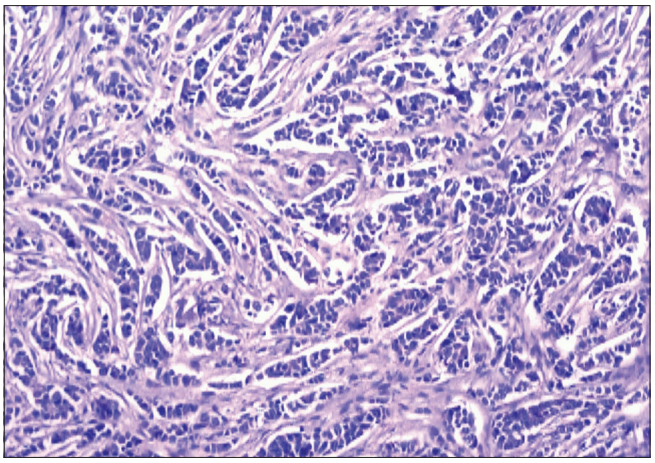

Figure 1: Luminal A subtype (H and E, ×100)

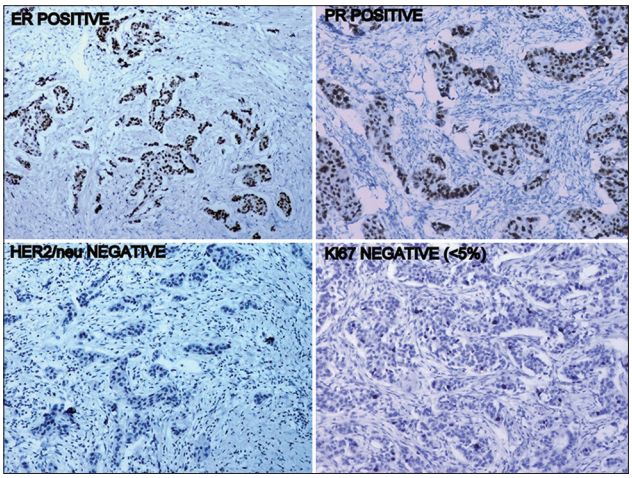

Figure 2: Luminal A subtype (immunohistochemical profile: ER, ×40; PR, ×100; Her2, ×100; Ki-67, ×100)

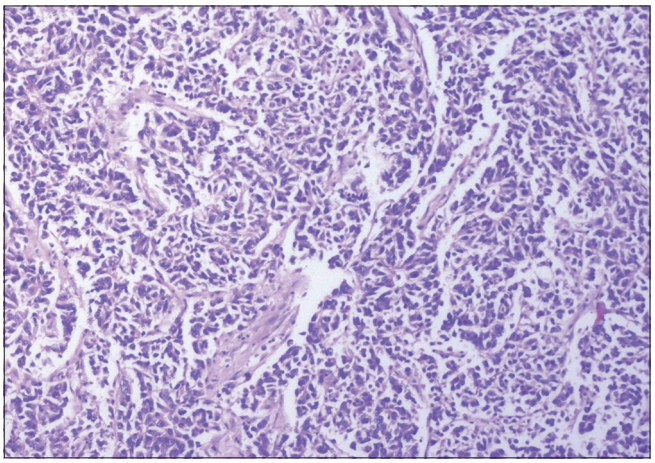

Figure 3: Luminal B human epidermal growth factor receptor 2-negative subtype (H and E, ×100)

subtypes were more common among postmenopausal patients. However, the association between IHC subtypes and age and menopausal status was not statistically significant (ANOVA test $F = 1.006$, $P = 0.411$ and $\chi^2 = 6.008$ $P = 0.199$, respectively).

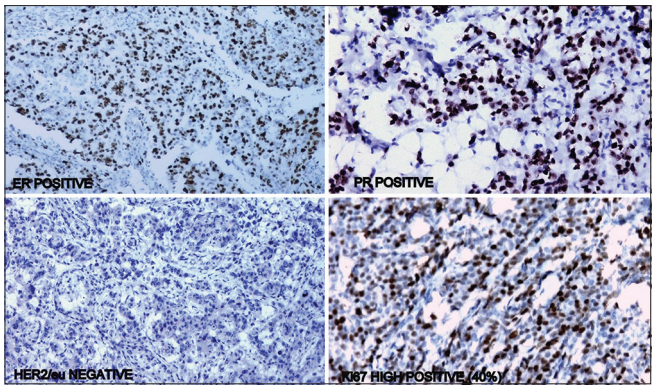

Figure 4: Luminal B human epidermal growth factor receptor 2-negative subtype (immunohistochemical profile: ER, ×100; PR, ×200; Her2, ×100; Ki-67, ×200)

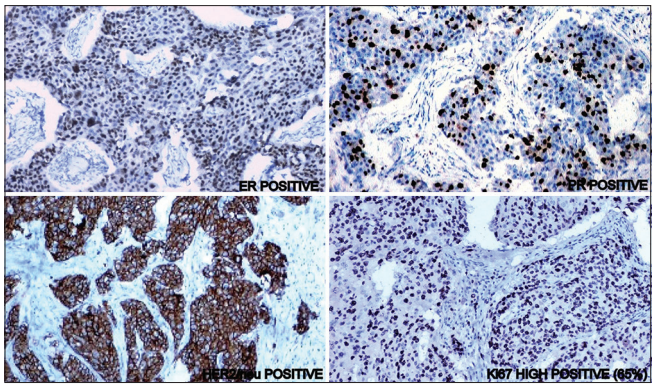

Figure 6: Luminal B human epidermal growth factor receptor 2-positive subtype (immunohistochemical profile: ER, ×100; PR, ×100; Her2, ×100; Ki-67, ×100)

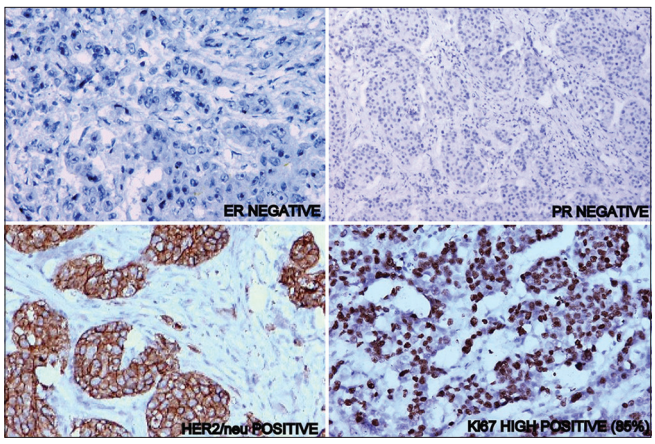

Figure 8: Human epidermal growth factor receptor 2/neu subtype (immunohistochemical profile: ER, ×200; PR, ×100; Her2, ×100; Ki-67, ×200)

Average tumor size was maximum in basal-like (3.9 cm) followed by Her2/neu+ subtype (3.5 cm) and was lowest in luminal A with an average size of 2.5 cm. There was no statistically significant difference in distribution of tumor size among various IHC subtypes (χ^2 = 8.575; P = 0.379; ANOVA test F = 2.386, P = 0.059). However, *post hoc* test revealed a statistically significant difference of mean tumor size between basal-like and luminal A subtype (*post hoc* test 3.9 vs. 2.5, P = 0.042).

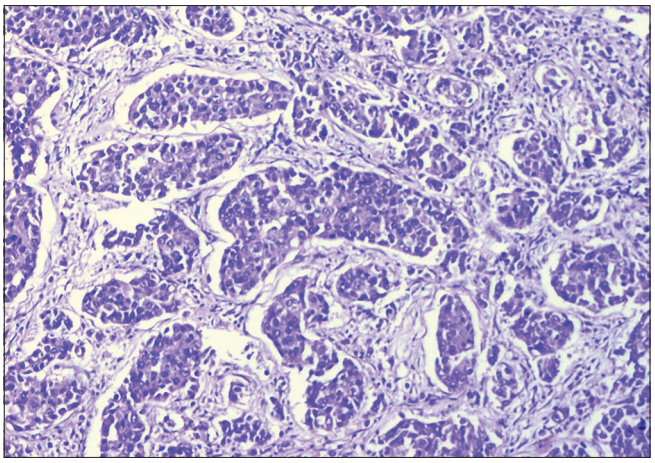

Figure 5: Luminal B human epidermal growth factor receptor 2-positive subtype (H and E, ×100)

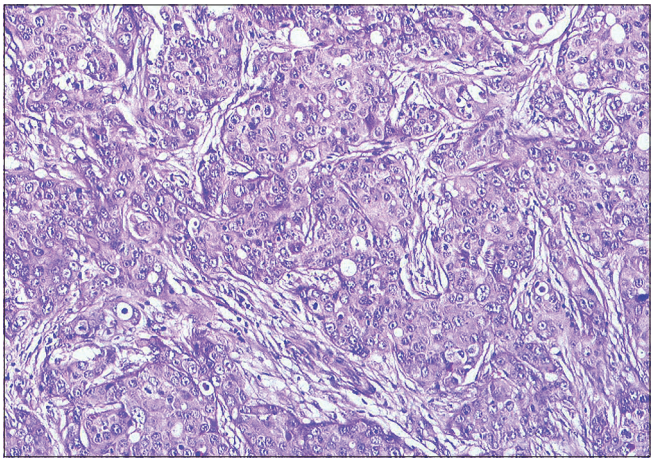

Figure 7: Human epidermal growth factor receptor 2/neu subtype (H and E, ×100)

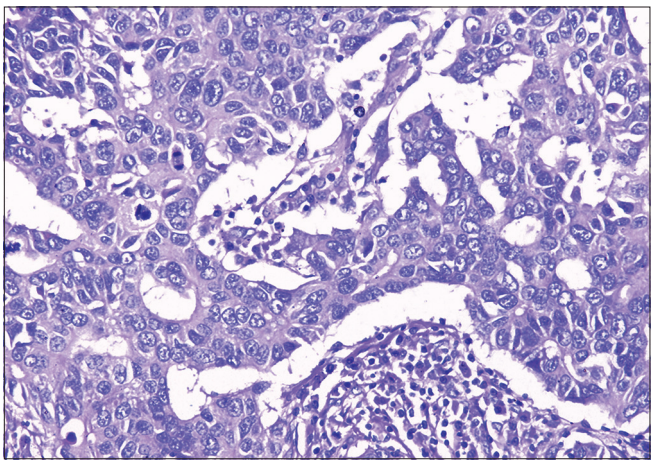

Figure 9: Basal-like subtype (H and E, ×200)

Basal-like subtype had the highest percentage of node-negative cases (50%) followed by luminal A and

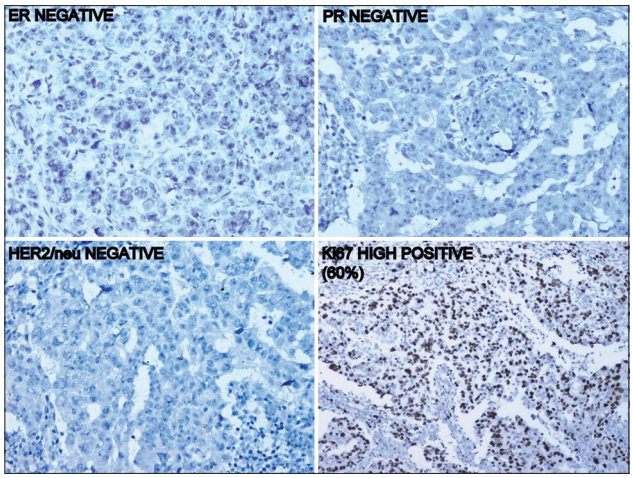

Figure 10: Basal-like subtype (immunohistochemical profile: ER, ×200; PR, ×200; Her2, ×200; Ki-67, ×100)

luminal B Her2− (38.1% and 28.3%, respectively). The association was statistically not significant (χ^2 = 9.244; P = 0.322). However, on stratification of cases on the basis of Her2/neu expression only, a statistically significant direct association was observed between Her2/neu expression and lymph node involvement (χ^2 = 4.125; P = 0.042).

The majority of cases in luminal A (71.4%) belonged to Grade I, whereas the majority of basal-like subtype (95%) and Her2/neu+ (77%) were of higher grade (II and III). The association of grade with different IHC subtypes was found to be statistically HS (χ^2 = 35.521; P < 0.001). Her2/neu+ subtype had highest number of cases belonging to poor prognostic group (46.1%), whereas luminal A had highest percentage of cases (38.1%) belonging to good prognostic group. However, when cases were stratified according to NPI, the association did not come out to be statistically significant (χ^2 = 15.036; P = 0.058).

We found a statistically significant association between Ki-67 LI and various IHC subtypes (χ^2 = 42.974; P < 0.001). Basal-like subtype had maximum average Ki-67 LI of 44.2%, followed by Her2/neu+ and luminal B Her2− with values of 27.5% and 24.1%. Luminal A had lowest average Ki-67 LI of 4.8% with a maximum number of cases (71.5%) being Ki-67 negative.

To summarize, a statistical significant association of various IHC subtypes was found with histologic grade, tumor size, and Ki-67, while the association was not significant with menopausal status, tumor size, lymph node involvement, and NPI [Table 2].

DISCUSSION

Breast cancer is characterized by genetic heterogeneity, exhibiting a wide variety of clinical presentations and of disease aggressiveness in different patients and ethnic populations and poses a major challenge in diagnosis and treatment. Molecular classification of breast cancers is useful for clinical management and is superior to the WHO classification in short-term prognostic value. Although IHC-based assays do not provide as much biological insight into tumor biology as gene-based ones do, they are increasingly used as a surrogate for molecular gene profiling since they allow classification of tumors at affordable costs and in the absence of fresh tissue specimens.[6]

IHC classification has prognostic and therapeutic implications, is readily available, and has the unique ability to provide comprehensive, and increasingly, quantitative multiplexed analysis of "gene expression" that can be directly linked to histology and all its subtleties.[16]

New approaches of combining established markers with novel factors are currently under evaluation. One of these is an immunopanel of ER, PR, Her2/neu, and Ki-67, whose ability to distinguish between luminal A and B subtypes in a similar manner as the original fifty-gene signature has been shown.[9] A recent finding from a study suggested that an IHC panel of just four frequently used markers is at least as prognostic as the oncotype recurrence score (RS) which indicates the need for further studies comparing new methodology with established, less-expensive methodology.[17]

Luminal A subtype is the most common of all IHC subtypes and comprises mainly of low-grade carcinomas. Characterized by ER/PR positivity, it displays low proliferative index.[16] It has the most favorable prognosis among all subtypes.[11] Luminal B type shows high proliferative index (>14% as per St. Gallen's molecular classification[13]) which has been used to differentiate it from luminal A types.[16] Prognosis is better than other subtypes but worse than luminal A.[18] Basal-like cancers are called so because of the positivity for basal high-weight CKs and specific myoepithelial cells markers (CK5/6, CK17, Caveolin1, Calponin1, p63). They lack ER, PR, and Her2/neu expression (triple negative) while Ki-67 is high.[18] They carry worst prognosis being poorly differentiated and having chances of soft tissue and visceral relapse and central nervous system metastasis.[19] However, they are less likely to have lymphomatous spread.[6,7] Her2/neu subtypes histologically corresponding to very aggressive high-grade ductal NOS carcinomas with poor prognosis but respond well to the humanized monoclonal antibodies against Her2/neu or Her2/neu tyrosine kinase inhibitors (trastuzumab). They are ER- and PR-negative tumors with a high Ki-67 positivity.[18]

The aim of our study was to classify breast carcinoma into various IHC subtypes and study their correlation with various other established clinicopathologic prognostic

Table 2: Correlation of different immunohistochemical subtypes with various clinicopathologic parameters

Clinicopathologic parameters	Luminal A	Luminal B		Her2/neu+	Basal-like	*P*
		Her2–	Her2+			
Number of cases (75)	21/75	14/75	7/75	13/75	20/75	
Age (years)						
Range	35-78	40-70	30-65	40-61	32-70	0.411
Mean (SD)	50.10	51.29	42.71	49.54	48.50	
Menopausal status						
Premenopausal	12/21	5/14	6/7	6/13	8/20	0.199
Postmenopausal	9/21	9/14	1/7	7/13	12/20	
Histology						
Ductal	18/21	13/14	6/7	11/13	18/20	-
Lobular	1/21	-	1/7	-	-	
Medullary	-	1/14	-	2/13	1/20	
Mucinous	1/21	-	-	-	-	
Secretory	1/21	-	-	-	-	
Metaplastic	-	-	-	-	1/20	
Tumor size (cm)						
<2	6/21	3/14	3/7	2/13	2/20	0.379
2-5	15/21	10/14	3/7	9/13	14/20	Luminal A versus basal: 0.042
>5	-	1/14	1/7	2/13	4/20	
Histologic grade						
Grade I	15/21	-	4/7	3/13	1/20	<0.001
Grade II	6/21	12/14	2/7	6/13	13/20	
Grade III	-	2/14	1/7	4/13	6/20	
Lymph node status						
Stage I	8/21	4/14	1/7	2/13	10/20	0.322
Stage II	3/21	5/14	2/7	5/13	6/20	
Stage III	10/21	5/14	4/7	6/13	4/20	
NPI						
Good prognostic group	8/21	1/14	1/7	1/13	1/20	0.58
Moderate prognostic group	9/21	8/14	5/7	6/13	15/20	
Poor prognostic group	4/21	5/14	1/7	6/13	4/20	
Ki-67 labeling index						
Average (%)	4.8	24.1	20.1	27.5	44.2	<0.001
Negative	15/21	-	2/7	2/13	1/20	
Positive	6/21	7/14	2/7	5/13	3/20	
High positive	-	7/14	3/7	6/13	16/20	

NPI: Nottingham prognostic index, SD: Standard deviation, Her2: Human epidermal growth factor receptor 2

parameters in North Indian population. All the cases in our study were divided into five IHC subtypes on the basis of ER, PR, Her2/neu, and Ki-67 expression as per St. Gallen guidelines.[13] Luminal A formed the major subtype in our study as in other studies in the literature.[5-8,11,19-21] Basal-like tumors constituted a larger group in our study. However, in a population-based study carried out by Carey *et al*., 39% of the premenopausal African American cases of breast carcinoma were basal-like and their higher prevalence could be because of strong influence of race and menopausal status and suggest that these tumors have a distinct etiology.[7] Her2/neu+ cases also represented a higher percentage in a study on Indian population by Munjal *et al*.[11] Similarly, a higher percentage of basal and Her2/neu+ subgroup in our study can be attributed to geographical and racial differences as none of these studies has been carried out in Southeast Asian settings.

All the IHC subtypes in our study predominantly comprised infiltrating ductal carcinoma with little variation and we could not categorize or compare the IHC subtypes by histologic group. However, a significant difference regarding histologic group among molecular subtypes was observed by Spitale *et al*.[6] All reported cases of metaplastic/anaplastic carcinoma with unfavorable prognosis were BCL cases in their study. Mean age in luminal B Her2+ subtype although was less 42.7 years than other subtypes in our study, but not statistically significant. Munjal *et al*. found that luminal A cases were significantly older than other subtypes.[11] Mean age in our study was lower than many of the studies in literature.[5-9,19-21] This could be attributed to racial and geographical variation. Small sample size of the study may be another factor contributing to the difference. Considering menopausal status, luminal B Her2–, Her2/neu+, and basal-like subtypes were more frequent in postmenopausal patients (64.3%, 53.9%, and 60%, respectively), while luminal A and luminal B Her2+ cases were more frequently seen in premenopausal patients (57.1 and 85.7%). However, statistically no significant association with menopausal status was observed as in various studies[7,11,19] while some of the other studies revealed a significant association.[5,6,8,20]

The highest proportion of small-sized tumors was seen in luminal B Her2+ (42.9%) and luminal A type (28.6%). Larger tumor (>5 cm) was more frequently observed in

basal-like (20%) and Her2/neu+ subtype (15.4%). Chi-square test and ANOVA did not reveal any significant difference of tumor size among various IHC subtypes. However, *post hoc* test revealed a pair-wise significant difference of mean tumor size between basal and luminal A subtype (3.9 vs. 2.5; $P = 0.042$). The association between tumor size and IHC subtypes was not found to be statistically significant in studies by Piñero-Madrona *et al*. and Munjal *et al*.[8,11] However, various other studies observed a statistically significant association between the two.[6,20,21] Significant differences among IHC subtypes were observed regarding histologic grade ($P < 0.0001$). Statistically significant association of histologic grade with various IHC subtypes is well in accordance with all the previous studies.[5-9,11,19-21]

Although the association between IHC subtypes and axillary lymph node status was not statistically significant, we did observe the highest percentage of negative lymph node cases in BCL (50%) and luminal A (38%) subtypes. In contrast, the majority of Her2/neu+ patients were lymph node-positive. Both our results are well in accordance with most of the previous studies.[6-8,20,21] Blows *et al*. and Zaha *et al*., however, have found basal type more frequently associated with lymph node metastases.[5,19] When we compared lymph node involvement in Her2+ subtypes (luminal B Her2+ and Her2/neu+) and Her2− subtypes (luminal A, luminal B Her2−, and BCL), we found a statistically significant association between Her2 positivity and lymph node metastasis. Similar results were observed by Spitale *et al*.[6]

The correlation of Ki-67 LI with different IHC subtypes was found to be statistically significant. The majority of the luminal A tumors being well differentiated were Ki-67-negative (71.4%) while 84.6% of Her2/neu+ and 95% of Basal subtype were positive. A very few have seen the expression of Ki-67 in different IHC subtypes, and our results were well in accordance with them.[6,8] The IHC subtypes differed significantly by MBR grade and Ki-67 proliferative index.

When IHC subtypes were compared with respect to age at diagnosis, menopausal status, and lymph node involvement, no significant difference was observed. However, we found a statistically significant association of Her2/neu expression with lymph node involvement ($P = 0.042$).

CONCLUSION

We made an attempt to characterize subtypes of breast cancer using IHC markers and have reached a fair conclusion that IHC classification is simple, informative, and fairly discriminative between various subtypes. The results obtained seem to confirm a significant difference of clinicopathologic characteristics in various IHC subtypes. However, our data need to be interpreted with some caution due to following reasons: First, our results were at slight variance from some of the Western studies in literature. This could be due to the influence of environmental and socioeconomic factors in the observed distribution of breast cancer subtype. Second, due to small sample size of the study, we could not find a significant statistical correlation in some of the parameters despite the consonance of results with available literature. Third, due to a short follow-up and unavailability of some of the clinical details, we were unable to provide correlative data between IHC subtypes, their clinical behavior, and the survival information.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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