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Study of Molecular Classes of Breast Cancer using Immunohistochemical Surrogate Markers in Correlation with Other Prognostic Parameters

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Abstract

Context: The classification of breast cancers into subgroups on the basis of gene expression patterns is often regarded as the gold standard, but its use remains limited due to the expense involved. Consequently there is interest in using Immunohistochemical (IHC) markers to classify tumors into subtypes that are surrogates for those based on gene-expression profiling. *Aims:* To classify molecular subtypes of breast carcinomas based on the surrogate IHC markers and to evaluate the correlation between these subtypes and other clinical parameters *Settings and Design:* The study was done from January 2014 to June 2016 on 70 cases of modified radical mastectomy specimens diagnosed as invasive breast cancer on histopathological examination. *Methods and Material:* The Immunohistochemical evaluation of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2/neu) were done on formalin fixed paraffin embedded tissue sections and are compared with the age of the patients, histological grade and tumor stage. *Statistical analysis used:* Analysis of variance for continuous variables and chi-square test for categorical variables. *Results:* The surrogate classification consisted of 36 cases of luminal A, 16 cases of luminal B, 13 cases of triple negative/basal-like tumors and 05 cases of HER2/neu subtype. The subjects of luminal A subtype were older, had a well/moderately differentiated histological grade, presented with small tumor size, less number of lymph nodal metastasis and had Early stage breast cancer. Subjects with triple negative tumors were younger, had moderately/poorly differentiated histological grade, presented with large tumor size, high lymph nodal metastasis and advanced stage breast cancer. *Conclusions:* IHC markers of breast cancer can be used as surrogate markers for molecular classification and are comparable with other prognostic markers.

Keywords: Breast Carcinoma; Immunohistochemical Markers; Molecular Classification; Progesterone Receptor; Estrogen Receptor.

Introduction

Breast carcinoma is the most common cancer among women in the urban Indian population and second only to cervical cancer in the rural population based on cancer registry data [1, 2, 3]. The classification of breast cancers into subgroups on the basis of gene expression patterns in tumor tissue is often regarded as the gold standard but its widespread use remains limited primarily due to the expense and technical difficulty encountered. Consequently there is interest in using Immunohistochemical (IHC) markers to classify tumors into subtypes that are surrogates for those based on gene-expression profiling.

St Gallen Consensus [4] has proposed a simplified, four IHC based biomarker panel (ER, PR, HER2, Ki67) for the molecular classification, which can be used as a shorthand and convenient approximation of intrinsic molecular subtypes of breast cancer. It has been proposed that if reliable Ki67 labeling index is not available, some alternative measure of proliferation like histological grade (G) may be used in making distinction between Luminal A and

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Luminal B subtypes (Goldhirsch et al., 2011) [4]. The surrogate IHC markers for molecular breast cancer subtypes have therefore emerged as a practical clinical tool to approximate molecular classification in breast cancer patients in the developing countries.

In this study breast cancer is classified into four groups based on IHC profile ER/PR and HER2/neu expression, positive (+) and/or negative (-). The groups are:

1. ER+/PR+, HER2/neu-; ER-/PR+, HER2/neu-; ER+/PR-, HER2/neu- (Luminal A)
2. ER+/PR+, HER2/neu +; ER-/PR+, HER2/neu +; ER+/PR-, HER2/neu+ (Luminal B)
3. ER-/PR-, HER2/neu- (triple negative/ Basal- like)
4. ER-/PR-, HER2/neu+ (HER2/neu)

The IHC classification corresponds well with intrinsic gene expression micro array categorization [5,6,7] and is shown in the Table 1

Aim and Objectives

The aim of the study is to sub classify molecular subtypes of breast carcinomas based on the surrogate IHC markers and the objectives are to evaluate the correlation between these subtypes with histological type, grade, and age of the patient, size of the tumor, lymph node status and TNM stage.

Subjects and Methods

The present study was done in our department from January 2014 to June 2016. A total of 70 modified radical mastectomy specimens diagnosed as invasive breast cancer on histopathological examination by using conventional Hematoxylin & Eosin stained sections were studied in detail.

The clinical details of these 70 cases were noted. Immunohistochemical evaluation was done on formalin fixed paraffin embedded tissue sections by using ready to use FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor α, Clone EP1 for ER, FLEX Monoclonal Mouse Anti-Human Progesterone Receptor, Clone PgR 636 for PR and by using Monoclonal Mouse Anti-Human HER2-pY 1248, Phosphorylation site specific, Clone PN2A for expression of HER2/neu protein and the detection system used was a polymer.

Expression of these target antibodies (ER,PR & HER2/neu) were used to sub classify molecular subtypes of breast carcinomas and were compared with other prognostic parameters like patient's age, histological type, size of the tumor, microscopic grading of breast cancer by using Nottingham modification of the Bloom-Richardson system & lymph node status. IHC scoring for ER/PR was done by Allred scoring & for HER2/neu by DAKO score [8]. The scoring system and criteria used for ER/PR and HER2/neu protein over expression are shown in Tables 2 & 3 respectively.

Allred Scoring

Is a semi quantitative system that takes into consideration the proportion of positive cells (scored on a scale of 0-5) and that of staining intensity (scored on a scale of 0-3). The proportion and intensity were then summed to produce a total score. If the sum of proportion score and intensity score is 0-2 it is considered as negative and if it is 3-8, then it is considered as positive.

Table 1: Molecular subtypes of breast cancer using IHC as surrogate marker

Immunoprofile	Luminal A	Luminal B	Triple negative /Basal-like	HER2/neu
ER, PR HER2/neu	ER and/or PR+ HER2/neu-	ER and/or PR+ HER2/neu+	ER-, PR- HER2/neu-	ER-, PR- HER2/neu+

Table 2: The ER/PR scoring system and criteria

Proportion Score	Observation	Intensity Score	Observation
0	None	0	None
1	1%	1	Weak
2	1-10%	2	Intermediate
3	10-33%	3	Strong
4	33-66%		
5	66-100%		

Inclusion Criteria

Modified radical mastectomy specimens that were reported as invasive breast carcinoma on histopathological examination

Exclusion Criteria

Cases of invasive breast carcinoma that were diagnosed in lumpectomy and core needle biopsy specimens.

Statistical Analysis

Differences in subjects and tumor characteristics between the various breast cancer subtypes were analyzed using analysis of variance for continuous variables and chi-square test for categorical variables. A *P*-value of <0.05 was considered statistically significant.

Results

Of the 70 cases included in the present study, the most common histological variant was invasive ductal carcinoma NOS type (91%), followed by three cases of invasive papillary carcinoma (04%), two cases of mucinous carcinoma (03%) and one case of medullary carcinoma (02%) as shown in Table 4.

Of the 70 cases studied, the surrogate classification consisted of 36 cases (51%) of luminal A, 16 cases (23%) of luminal B, 13 cases (19%) of triple negative/Basal-like and 5 cases (07%) of HER2/neu tumors (Table 5).

The most common age group affected was in between 41-60 years that comprised 47% of the entire study group. As the age increased, the number of cases classified as Luminal A subtype also increased. Whereas for Luminal B, triple negative and for HER2/neu tumors, as the age increased the number of cases decreased (Table 6).

A higher percent of cases were in grade1. As the grade increased, the number of cases of Luminal A subtype decreased while triple negative and HER2/neu tumors increased (Table 7).

Most of the tumors were in stage I. For Luminal A subtype majority of the cases were in tumor stage I, while the number of cases in stage III were more in Triple negative and HER2/neu subtypes (Table 8).

Table 3: HER2/neu scoring system and criteria

Staining pattern	Score	HER2/neu protein over expression assessment
No staining or membrane staining observed in <10% of tumor cells	0	Negative
Faint or barely perceptible membrane stain in >10% of tumor cells	1+	Negative
Weak to moderate complete membrane staining in >10% of tumor cells	2+	Weakly positive
Strong complete membrane staining in >30% of tumor cells	3+	Strongly positive

Table 4: Histological variants among the cases studied

Histological variants	No. of cases
Invasive ductal carcinoma NOS type	64 (91%)
Invasive papillary carcinoma	03 (04%)
Medullary carcinoma	01 (02%)
Mucinous carcinoma	02 (03%)
Total no. of cases	70 (100%)

Table 5: Molecular classification of breast cancers using surrogate IHC markers

Luminal A	36 (51%)
Luminal B	16 (23%)
Triple negative /Basal-like tumors (TN)	13 (19%)
HER2/neu tumors	05 (07%)
Total no. of cases	70 (100%)

Table 6: Age distribution among the molecular classification of breast cancers

Age of the patient (in years)	Luminal A	Luminal B	TN	HER2/neu	No. of cases
21-30	--	--	01	--	01 (1.4%)
31-40	--	01	02	03(60%)	06 (8.6%)
41-50	05 (14%)	08	05	01(20%)	19 (27%)
51-60	08(22%)	04	02	--	14 (20%)
61-70	10(28%)	03	02	01(20%)	16 (23%)
71-80	13(36%)	--	01	--	14 (20%)
Total no. of cases	36	16	13	05	70

Table 7: Nottingham modification of the Bloom-Richardson system for tumor grading

Histological grade	Luminal A	Luminal B	Triple negative	HER2/neu	No. of cases
Grade 1	28 (78%)	07 (44%)	01 (8%)	--	36 (51%)
Grade 2	08 (22%)	08 (50%)	05 (38%)	02 (40%)	23 (33%)
Grade 3	--	01(06%)	07(54%)	03 (60%)	11 (16%)
Total no. of cases	36	16	13	05	70 (100%)

Table 8: TNM staging of the tumors included in the study

Stage	Luminal A	Luminal B	Triple negative	HER2/neu	No. of cases
I	28 (78%)	08 (50%)	--	--	36 (51%)
II	06 (16%)	05 (31%)	08 (62%)	02 (40%)	21 (32%)
III	02 (06%)	03 (19%)	05 (38%)	03 (60%)	13 (17%)
Total no. of cases	36	16	13	05	70 (100%)

Table 9: Baseline characteristics by tumor subtype

	Luminal A	Luminal B	Triple negative/ Basal-like	HER2/neu	P-value
Mean age (years)	63.6	51	48.5	42.6	0.00002
Cancer type					
Invasive ductal Carcinoma NOS type	34 (94%)	13(82%)	12 (92%)	05 (100%)	
Medullary carcinoma	--	--	01(08%)	--	
Mucinous carcinoma	01(03%)	01 (6%)	--	--	
Invasive papillary carcinoma	01(03%)	02 (12%)	--	--	
Histological grade					
Well differentiated (Grade 1)	28 (78%)	07 (44%)	01 (08%)	--	0.00001
Moderately differentiated (Grade2)	08 (22%)	08 (50%)	05 (38%)	02 (40%)	
Poorly differentiated (Grade 3)	--	01 (06%)	07 (54%)	03 (60%)	
Tumor size					
</= 2cm	29 (80%)	09 (56%)	--	--	0.0001
2-5cm	05 (14%)	06 (38%)	08 (62%)	05 (100%)	
>5cms	02 (06%)	01 (06%)	05 (38%)	--	
Lymph nodal metastasis	17%	38%	100%	100%	
1-3 positive nodes	04	03	08	03	0.0001
4-9 positive nodes	02	03	04	02	
>10 nodes	00	00	01	00	
Tumor stage					
I	28 (78%)	08 (50%)	00	00	0.0001
II	06 (16%)	05 (31%)	08 (62%)	02 (40%)	
III	02 (06%)	03 (19%)	05 (38%)	03 (60%)	

Differences in baseline characteristics between 4 subtypes are presented in Table 9.

Subjects with luminal A subtype are older ($p<0.05$), had a well/moderately differentiated histological grade ($p<0.05$), presented with small tumor size ($p<0.05$). They had less number of metastatic axillary lymph nodal deposits ($p<0.05$) and had early stage breast cancer ($p<0.05$). Subjects with triple negative tumors are younger ($p<0.05$), had moderately/poorly differentiated histological grade ($p<0.05$) and presented with large tumor size ($p<0.05$). They had high lymph nodal metastasis ($p<0.05$) and had advanced stage breast cancer ($p<0.05$).

Discussion

The classification of breast cancers into subgroups on the basis of gene expression patterns in tumor tissue is often regarded as the gold standard, but widespread use of gene-expression profiling in either the clinical or the research setting remains limited. Lack of widespread use of expression profiles are primarily due to the financial constraints and technical difficulties encountered. Hence, there is an interest in using IHC markers to classify tumors into subtypes that are used as alternative for gene-expression profiling. The IHC-based classification

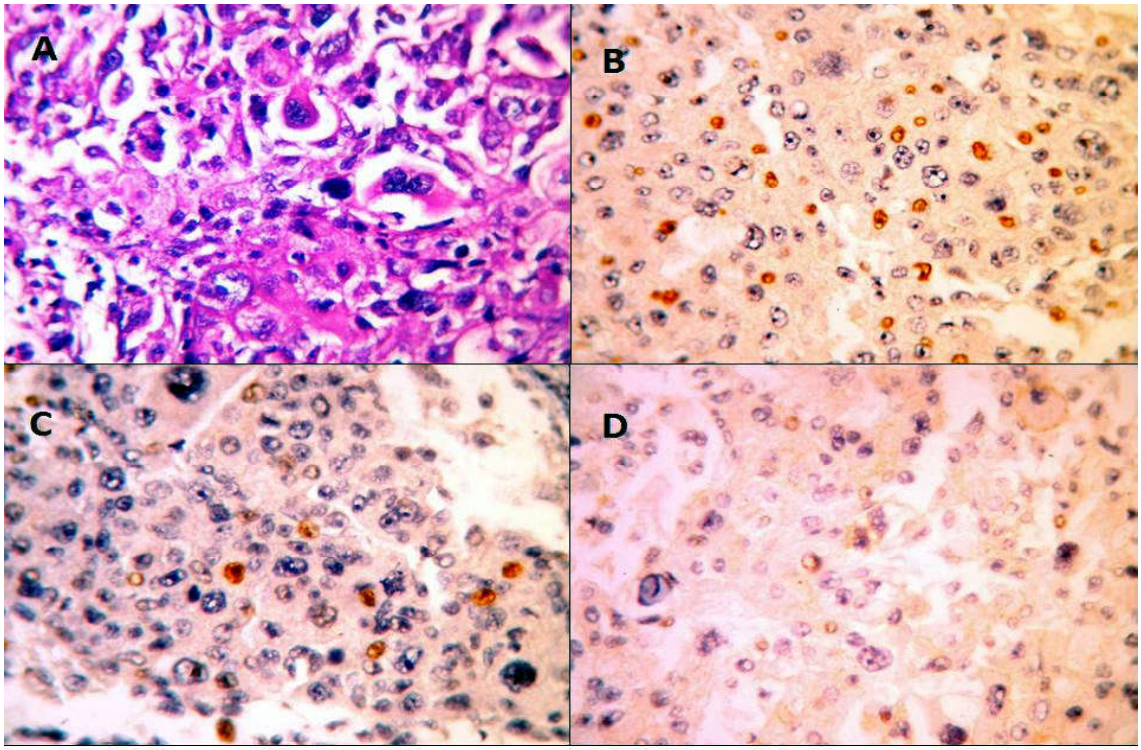

Fig. 1: Luminal A molecular subtype. **A.** Invasive ductal carcinoma NOS type (H&E x 400) **B.** ER positivity in tumor cells (IHC stain, ER x 400) **C.** PR positivity in tumor cells (IHC stain, PR x 400) **D.** HER2 negativity in tumor cells (IHC stain, HER2 x 400)

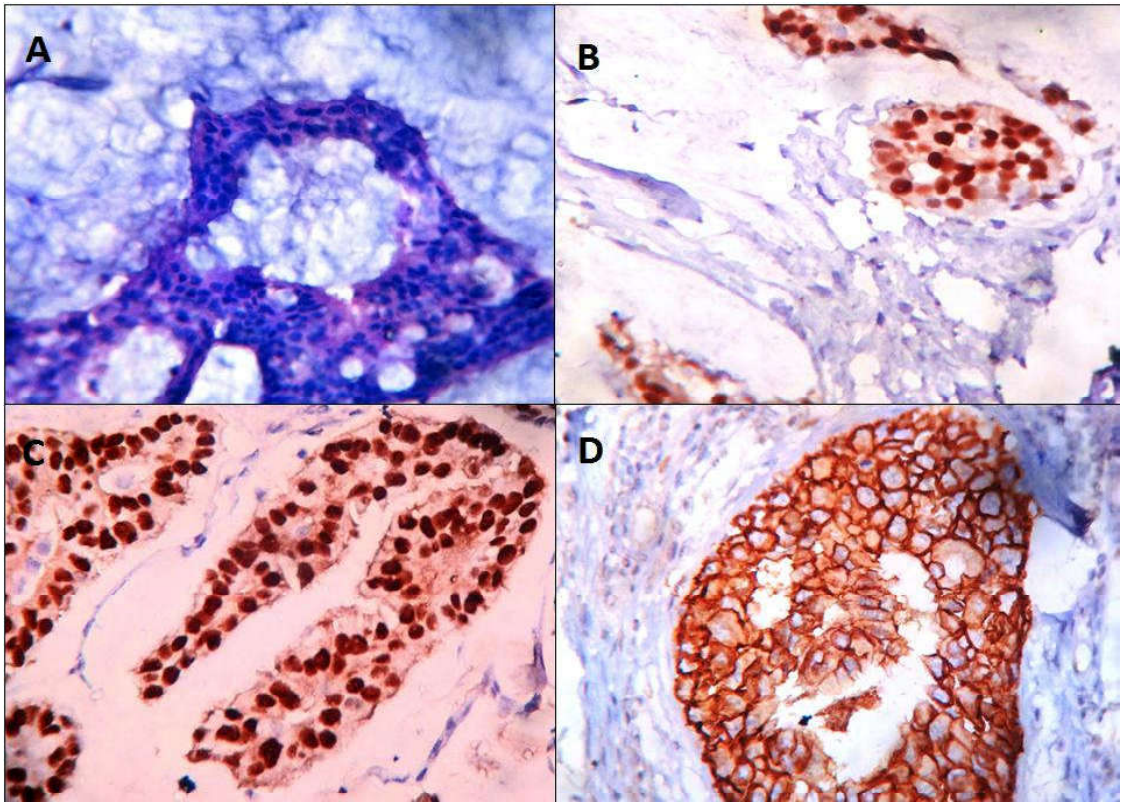

Fig. 2: Luminal B molecular subtype **A.** Mucinous carcinoma (H&E x400) **B.** ER positivity in tumor cells (IHC stain, ER x 400) **C.** PR positivity in tumor cells (IHC stain, PR x 400) **D.** HER2 positivity in tumor cells (IHC stain, HER2 x 400)

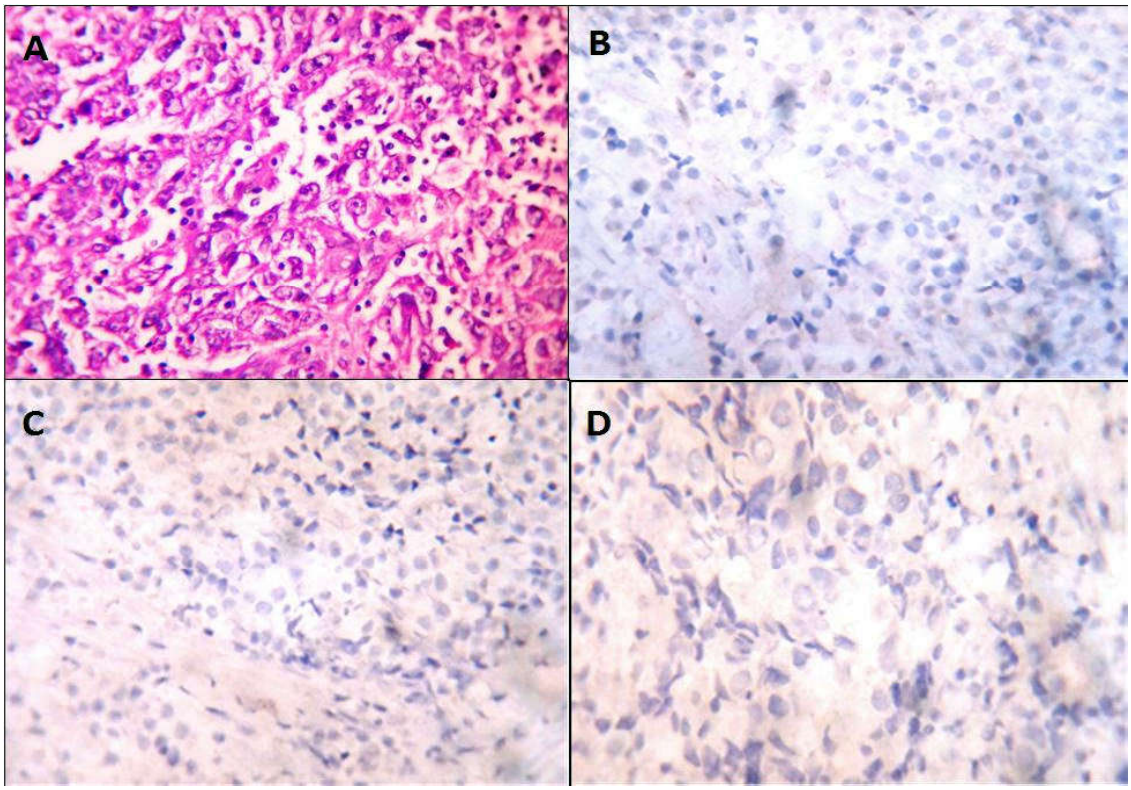

Fig. 3: Triple negative/ Basal like molecular subtype **A.** Medullary carcinoma (H&E x400) **B.** ER negativity in tumor cells (IHC stain, ER x 400) **C.** PR negativity in tumor cells (IHC stain, PR x 400) **D.** HER2 negativity in tumor cells (IHC stain, HER2 x 400)

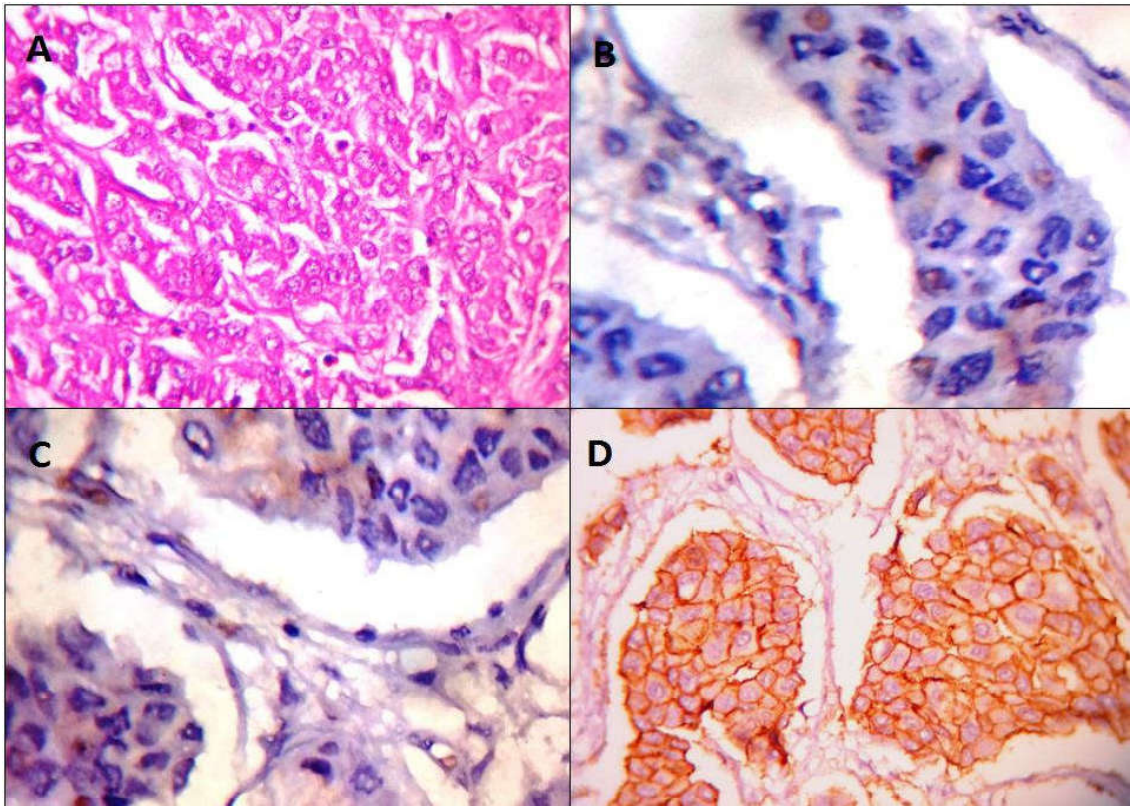

Fig. 4: HER2/Neu molecular subtype **A.** Invasive ductal Carcinoma NOS type (H& E x 400) **B.** ER negativity in tumor cells (IHC stain, ER x 400) **C.** PR negativity in tumor cells (IHC stain, PR x 400) **D.** HER2 positivity in tumor cells (IHC stain, HER2 x 400)

Table 10: Differences in the baseline features of breast cancer subtype studied

	Luminal A	Luminal B	Triple negative	HER2/neu
No. of cases	36 (51%)	16 (23%)	13 (19%)	05 (07%)
Histological variants				
Invasive ductal				
Carcinoma NOS type	34 (94%)	13(82%)	12 (92%)	05 (100%)
Medullary carcinoma	--	--	01(08%)	--
Mucinous carcinoma	01(03%)	01 (6%)	--	--
Invasive papillary carcinoma	01(03%)	02 (12%)	--	--
Mean age (years)	63.6	51	48.5	42.6
Histological grade				
Well differentiated (Grade 1)				
Moderately differentiated	28 (78%)	07 (44%)	01 (08%)	--
(Grade2)	08 (22%)	08 (50%)	05 (38%)	02 (40%)
Poorly differentiated	--	01 (06%)	07 (54%)	03 (60%)
(Grade 3)				
Mean tumor size in cm	2.08	2.75	5.01	3.5
No. of cases with lymph nodal metastasis	06 (17%)	06 (38%)	13 (100%)	05 (100%)
Tumor stage				
I	28 (78%)	08 (50%)	00	00
II	06 (16%)	05 (31%)	08 (62%)	02 (40%)
III	02 (06%)	03 (19%)	05 (38%)	03 (60%)

systems are useful in clinical practice, especially when fresh tissue is not available, and has been shown to correlate well with intrinsic classification using gene expression micro arrays [9].

In the present study, luminal A subtype constituted the majority (51%) of the cases, most of them being invasive ductal carcinoma NOS type. Luminal A subtype also included a single case each of invasive papillary carcinoma and mucinous carcinoma. The mean age of presentation of luminal subtype A was 63.6 years. Most of these cases (78%) showed low histological grade i.e.; grade 1. The mean tumor size was 2.08cms. Only 6 cases (17%) out of 36 cases of luminal A subtype showed axillary lymph nodal metastasis and most of them were in stage I (78%).

The second majority subset was Luminal B subtype comprising 23% of the cases which included two cases of invasive papillary carcinoma and a single case of mucinous carcinoma. The rest of them were invasive ductal carcinoma, NOS type. The mean age of presentation was 51 years. Most of these cases (50%) were graded as grade 2 tumors. The mean tumor size was 2.75cms. 38% of these tumors showed axillary lymph nodal metastasis and most of them (50%) were stage I tumors followed by stage II and stage III tumors.

Triple negative/basal-like tumors comprised the third large group constituting 19% of the total cases studied that also included a single case of medullary carcinoma. The remaining being high grade invasive ductal carcinoma, NOS type. The mean age of presentation was 48.5 years. Most of these cases (54%) were graded as grade 3 tumors. The mean tumor size was 5.01cms. All these cases showed axillary lymph nodal metastasis and most of them (62%) were in stage II followed by stage III tumors. None of these cases were noted in stage I.

HER2/neu subtype constituted the least common subtype in the present study comprising only 7% of the study population. The mean age of presentation was 42.6 years. Most of these cases were grade 3 tumors (60%)followed by grade 2 (40%). None of these were grade 1 tumors. The mean tumor size was 3.5cms. Axillary lymph nodal metastasis was observed in all the cases of this tumor subtype and most of them were in stage III (60%) and the remaining were stage II tumors. None of these cases were in stage I when detected.

All these observations are presented in Table 10.

Based on the above observations, we concluded that Luminal A subtype of breast cancer carried very good clinical and pathological prognosis. Triple negative/ basal-like and HER2/neu tumors carried poor clinical and pathological prognosis. Our findings were similar to those of the studies done by Carey LA et al [10], Dent R et al [11] and Azizun-Nisa et al [12] . According to the studies done by some authors [13, 14] no significant correlation was observed between these molecular sub types and certain prognostic parameters.

Conclusion

By using the surrogate IHC markers for molecular classification of breast cancer, we concluded that luminal A subtype had good prognosis and triple negative subtype had the worst prognosis which were similar to those of molecular subtypes of breast cancers that were classified by using tissue micro arrays. Thus IHC can be used as surrogate markers for molecular classification of breast carcinomas, especially in the developing countries where the health care budgets are limited and since ER/PR and HER2/neu testing is already widely available at a reasonable cost. Additional ongoing efforts should be directed at standardization of current testing methods and development of more reliable and reproducible testing.

Key Messages

IHC markers of breast cancer can be used as surrogate markers for molecular classification. They are therapeutically informative, cost effective and comparable to other prognostic markers.

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