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Original Article

Pathology Section

Histopathological Variants and Molecular Subtypes of Carcinoma Breast in a Tertiary Care Centre, Kerala, India: A Cross-sectional Study

ANJALI RAJU[1], JAYASREE RAMAN[2], KAVITHA RAVI[3]

ABSTRACT

Introduction: Breast cancer is the second leading cause of cancer deaths in women after lung cancer. Breast cancer survival varies by racial and ethnic factors, stage at diagnosis, tumour grade, molecular subtypes and the treatment received. Molecular subtyping provides prognostic and predictive information about the risk of recurrence and is an essential tool in formulating guidelines in therapy.

Aim: To identify the histopathological variants of Carcinoma (Ca) breast in women and to determine the various molecular subtypes by Immunohistochemistry (IHC).

Materials and Methods: A cross-sectional study from January 2019 to December 2020 was done on 100 cases of invasive carcinoma breast at the Department of Pathology in a tertiary care center of Government Medical College, Ernakulam, Kerala. IHC was done on paraffin processed tissue sections of tumour using anti-Oestrogen Receptor (ER), anti-Progesterone Receptor (PR), anti-Human Epidermal growth factor Receptor 2 (HER2/neu) and Ki-67 antibodies. Molecular subtypes of Luminal-A, Luminal-B, HER2 enriched and triple negative (basal-like) were determined. The association between molecular subtypes and tumour grade, size, stage was analysed using IBM Statistical Package for the Social Sciences (SPSS) version 21.0 software. Chi-square test used for categorical variables, p-value <0.05 assumed to be significant.

Results: Total 100 female cases of invasive carcinoma breast with mean age 49.3±12.2 years were included. Histologic subtypes of carcinoma were: Invasive Ductal Carcinoma (IDC) of No Special Type (NST) (89%), Invasive Lobular Carcinoma (ILC) (1%), Invasive ductal with Lobular carcinoma (IDC-L) (1%), metaplastic (2%), papillary (4%), IDC with medullary like features (3%). Tumour size was pT1 in 27%, pT2 in 38%, pT3 in 33%, pT4 in 2%. Tumour grades were: grade-I (28%), grade-II (29%) and grade-III (43%). Lymph node metastasis was seen in 52% cases. Positive expression of Oestrogen (ER) in 46%, Progesterone (PR) in 38%, HER2/neu in 23% and low Ki-67 labeling index (<14%) in 32% cases were observed. The molecular subtypes were Luminal-A (32%), Luminal-B (14%), HER2 enriched (16%) and triple negative (38%) in the present study.

Conclusion: The most common molecular subtype was triple negative. Luminal-A subtype was associated with lower histologic grade and non luminal subtypes were associated with higher histologic grades. To determine molecular subtypes, IHC is useful as a surrogate for molecular testing.

Keywords: Biomarker breast, Breast cancer, Luminal like, Triple negative

INTRODUCTION

Female breast cancer has surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases [1]. In India, it has been ranked as number one cancer among females with age adjusted rate of 25.8 per 100,000 women and mortality 12.7 per 100,000 women [2]. Breast cancer is diverse in terms of histopathological and molecular characteristics, metastatic patterns and response to therapy. Refined pathological and molecular subtyping allows clinicians to offer individualised targeted therapy which improves the outcome [3].

Gene expression profile of ER, PR and HER2/neu has been used to describe the intrinsic molecular subtypes of breast cancer. It provides predictive information on the potential responsiveness of tumours to therapeutic modalities and correlate with prognosis [4]. The 12th St. Gallen International Breast Cancer Conference (2011) Expert Panel adopted a classification for therapeutic purposes based on molecular biological subtypes of primary tumours [5]. The distinct molecular subtypes described are Luminal-A, Luminal-B (HER2-), Luminal-B (HER2+), HER2 enriched and basal-like (triple negative) subtypes, the categorisation can be performed using IHC surrogate marker-based analysis [6].

ER positivity is a strong predictive factor for response to hormone therapies like Tamoxifen (selective ER modulator) and aromatase inhibitors (suppress the production of oestrogen) [5]. PR is an oestrogen-regulated gene and its expression indicates that ER pathway is functioning [7]. HER2/neu is an oncogene, which belongs to the family of epidermal growth factor receptors. Assessment of HER2 gene amplification by ISH (in situ hybridisation technique) or protein overexpression by IHC is used in targeted therapy [8]. Assessment of triple markers ER, PR, and HER2/neu, and proliferative activity based on Ki-67 score have become essential requirements for the oncologists in treatment of breast cancer [9].

Molecular Subtypes: Luminal-A like tumours comprises approximately 60% of invasive breast carcinomas, express Luminal-cytokeratins, high expression of hormone receptors, are HER2 negative and have low proliferation rate. Respond to endocrine therapy, associated with better prognosis [10].

Luminal-B like tumours seen in about 10% of invasive breast cancers, express Luminal-cytokeratins, show weak expression of PR. ER positive, PR low positive, HER2 expression variable, Ki-67 index high. Luminal-B tends to be higher histologic grade than luminal-A. They respond to endocrine therapy, show variable response to chemotherapy [10].

HER2- enriched tumours comprises about 15% of invasive breast cancers, show high expression of HER2 and other genes in

amplicon on 17q12, low expression of ER, PR. Mutation of TP53 is common, more likely to be high grade, node positive and have poor prognosis. They respond to HER2 targeted therapies [11].

Basal-like (Triple negative) tumours comprises 15% of invasive breast cancers, show high expression of basal cytokeratins and low expression of HER2 related genes. Most are ER/PR and HER2 negative and show high Ki-67 index. TP53 mutation is common, have poor prognosis and show no response to endocrine therapy or trastuzumab (herceptin). Platinum-based chemotherapy and Poly (ADP-ribose) polymerases (PARPs) inhibitors are indicated [11].

The 8th edition of the primary Tumour, lymph Node, and Metastasis (TNM) classification of the American Joint Committee on Cancer has incorporated prognostic influence of tumour grade, hormone receptor expression, and HER2 amplification into the staging system and assigned "Prognostic stage groups" to the tumours [12]. Tumour biomarkers and multigene panel assays, Genetic tests like Oncotype DX or MammaPrint are validated tests to predict the recurrence of disease [12]. This study was aimed to analyse the proportion of molecular subtypes of breast cancers in our setting and to study the association of biomarkers with histopathological parameters.

MATERIALS AND METHODS

A cross-sectional study was conducted on resection specimens of breast cancer in women, in the Department of Pathology in a tertiary center of Government Medical College, Ernakulam, in Kerala, during January 2019- December 2020. Institutional scientific committee and Ethics committee approval were obtained. (No. IEC-27/2019).

Inclusion criteria: Female breast cancer specimens of mastectomy, wide excision and breast lumpectomy were included.

Exclusion criteria: Trucut biopsies with inadequate tissue for IHC, in situ carcinoma and post chemotherapy, Ductal Carcinoma In Situ (DCIS) and postchemotherapy/postlumpectomy resections with no residual tumour were excluded.

Sample size calculation: It was calculated based on the study by Tiwari N and Gupta P in which 56.2% patients had triple negative breast cancer [13]. Using the formula 3.84 pq/d^2, substituting the values as (p=56.2, q=43.8 and d=10), the minimum sample size calculated was 3.84x56.2x 43.8/100=95.

Study Procedure

Clinical details including age and clinical presentation were recorded in the proforma. Specimens of mastectomy and breast lump excisions were sampled as per the College of American Pathologists (CAP) protocol [14]. Tumour size, histopathological diagnosis, tumour grade, and lymph node metastases recorded. Histopathological tumour grading was done using Modified scarff Bloom Richardson (MBR) scoring [15]. The TNM staging was done as per the AJCC guidelines [12]. IHC staining was done manually for ER, PR, HER2/neu, and Ki-67 biomarkers. Tissue sections, 3-microns thick were taken on silane coated slides, antigen retrieval by heat induced epitope retrieval and tris-buffered saline at pH 9.0, incubated with primary antibody, antibody detection by Super Sensitive Polymer-HRP /DAB(Diamino-benzidine) IHC detection system. Positive internal and external controls for ER and PR, positive external controls for Ki-67 and HER2 neu were used. (The primary antibodies used were Rabbit mono ER-PR042, Rabbit mono PR-PR068, Rabbit mono HER2/neu PR047, Mouse mono Ki-67-PM096. Path in situ).

The staining pattern of ER, PR, and Ki-67 is confined to the nucleus. Proportional score (range of 0-5) based on a percentage of cells showing nuclear stain and intensity score (range of 0-3) based on the staining intensity were taken. A cut-off value of 1% positive cells used as the criteria for positivity in interpreting ER or PR tumour status. ER, PR staining quantified (range from 0-8) using the Allred score as the sum of the proportion score and intensity score. Allred score 0-2 was taken as negative, score 3-8 was considered positive [7]. Based on American Society of Clinical Oncology / College of American Pathologists (ASCO/CAP) guidelines for reporting of HER2/neu by IHC, positive 3+stain is circumferential membrane staining that is complete, intense in >10% of tumour cells. Weak to moderate complete membrane staining observed in >10% of tumour is 2+, incomplete faint membrane staining in >10% of the invasive tumour cell is 1+, incomplete membrane staining ≤10% of tumour cells/no staining is 0. Score 0-1+is negative, score 2+is equivocal, and score 3+is positive [8,14]. Ki-67 labelling index less than 14% was considered low [5]. To differentiate Luminal-B from Luminal-A, HER2 over expression, PR status negative or low and Ki-67 labelling index >14% was used [11].

Based on the positivity of markers, the four molecular subtypes were categorised as follows [11]:

- Luminal-A like: ER+, PR+, HER2/neu-, Low Ki-67 (less than 14%).
- Luminal-B like (HER2-): ER+, HER2 -, at least one of the following, Ki-67 index >14%/ or PR- or low
- Luminal-B like (HER 2 +): ER+, HER2 overexpressed or amplified, Ki67 index: any, PR any.
- HER2 enriched: HER2/neu+, ER-and PR-.
- Triple-negative: ER-, PR- and HER2/neu-.

Pathological characteristics of each case and molecular subtyping were compared for analysis.

STATISTICAL ANALYSIS

The statistical analysis was determined using the Pearson's Chi-square test. Significance was assumed at p-value<0.05. Data were entered in Microsoft Excel and analysed using the IBM SPSS version 21.0 software package. Descriptive analysis was carried out by frequency and proportion.

RESULTS

In the present study of 100 cases of breast cancer in women, the age of the patients ranged from 30 years to 80 years with the mean age 49.3±12.2 years. Maximum cases (28%) were in 41-50 years of age. Most of the cases (52%) were of right sided. Most patients (79%) presented with breast lumps. The tumour size ranged from 1.5 to 9 cm, 27% had tumour size <2 cm (pT1), 38% had 2 to 5 cm size (pT2), 33% had tumour size >5 cm (pT3), 2% tumours had skin involvement (pT4b). Histologic tumour grades (MBR) were grade-I in 28%, grade-II in 29% and grade-III in 43%cases. The demographic profile and clinical presentation of the cases are summarised in [Table/Fig-1].

Parameters		No. of cases	%
Age (in years)	30-40	8	8%
	41-50	28	28%
	51-60	25	25%
	61-70	26	26%
	71-80	13	13%
	Mean±SD	49.3±12.2	
Laterality	Right	52	52%
	Left	48	48%
Clinical presentation	Palpable Lump	79	79%
	Palpable lump, pain	1	1%
	Lump, Ulcer	1	1%
	Nipple Discharge	4	4%
	Nipple discharge, pain	1	1%
	Pain	13	13%
	Ulcer	1	1%

[Table/Fig-1]: Clinical parameters of female breast carcinoma (n=100).

The histological types encountered in our study were IDC breast of NST 89%, invasive papillary carcinoma (4%), invasive lobular carcinoma (1%), IDC with lobular carcinoma (1%), carcinoma with medullary features (3%) and metaplastic carcinoma (2%). Out of the 100 cases, 39% cancers were in stage IIIA.

The pathological characteristics of tumours are summarised in [Table/Fig-2].

Characteristics		%
Tumour size (cm)	<2 cm (pT1)	27%
	2-5 cm (pT2)	38%
	>5 cm (pT3)	33%
	Skin involvement (pT4b)	2%
Lymph node metastasis	Present	52%
Histologic type	IDC-NST	89%
	IDC with invasive lobular carcinoma (ILC)	1%
	Invasive lobular carcinoma	1%
	Metaplastic Ca	2%
	Invasive papillary Ca	4%
	IDC with medullary like features	3%
Tumour grade	MBR grade-I	28%
	MBR grade-II	29%
	MBR grade-III	43%
TNM stage	IA	17%
	IIA	19%
	IIB	23%
	IIIA	39%
	IIIB	2%

[Table/Fig-2]: Pathological characteristics of invasive breast carcinoma (N=100).

ER positivity in 46 cases (46%), PR positivity in 38 cases (38%) and HER2 positivity in 23 cases (23%) were observed. HER2 was negative in 75 cases (75%) and equivocal in 2 % cases. Ki-67 <14% was seen in 32%, Ki-67 >30% in 47%. The tumours were classified into molecular subtypes using protein expression patterns in IHC. The tumours were Luminal-A (32%), Luminal-B (14%), HER2 enriched (16%), and Triple negative (38%). Biomarker expression in 100 cases is as shown in [Table/Fig-3].

Biomarker status		No. cases	%
ER	ER+	46	46%
	ER-	54	56%
PR	PR+	38	38%
	PR-	62	62%
ER, PR	ER+/PR+	38	38%
	ER+/PR-	08	08%
HER2	Positive	23	23%
	Negative	75	75%
	Equivocal	2	2%
Ki-67 proliferation index	<14%	32	32%
	≥14-30%	21	21%
	>30%	47	47%
Molecular subtypes	Luminal-A	32	32%
	Luminal-B (HER2-)	7	14%
	Luminal-B (HER2+)	7	
	HER2 Enriched	16	16%
	Triple negative	38	38%

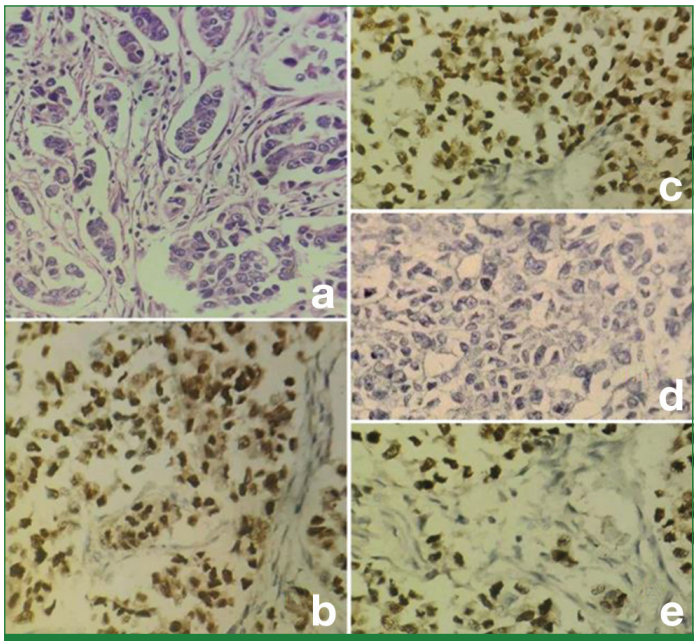
[Table/Fig-3]: Biomarker expression and molecular subtypes of breast carcinoma (N=100).
ER: Oestrogen; PR: Progesterone

The most common molecular subtype was triple negative (38%). The split up of morphology of triple negative cases were 33 cases of IDC-NST, three cases of IDC with medullary features and two cases of metaplastic carcinoma. Luminal-A subtypes (32%) were seen in IDC-NST in 28/32 cases and in papillary carcinoma (4/4). The histologic types and the molecular subtypes are shown in [Table/Fig-4].

Histologic type of carcinoma	Molecular subtypes				Total
	Luminal-A	Luminal-B	HER2 enriched	Triple negative	
IDC-NST	28 (87.5%)	13 (92.9%)	16 (100%)	32 (84.2%)	89 (89%)
Mixed-IDC and Invasive Lobular	0	0	0	1 (2.6%)	1 (1%)
Invasive lobular (ILC)	0	1 (7.1%)	0	0	1 (1%)
Metaplastic Ca	0	0	0	2 (5.3%)	2 (2%)
Invasive Papillary (IPC)	4 (12.5%)	0	0	0	4 (4%)
IDC with medullary features	0	0	0	3 (7.9%)	3 (3%)
Total	32 (100%)	14 (100%)	16 (100%)	38 (100%)	100 (100%)

[Table/Fig-4]: Histologic type and molecular subtypes of Ca Breast (n=100).

Photomicrographs of IHC staining of Luminal-B molecular subtype, showing ER positive, PR positive, HER2 negative and Ki-67 positivity >14% is shown in [Table/Fig-5a-e].



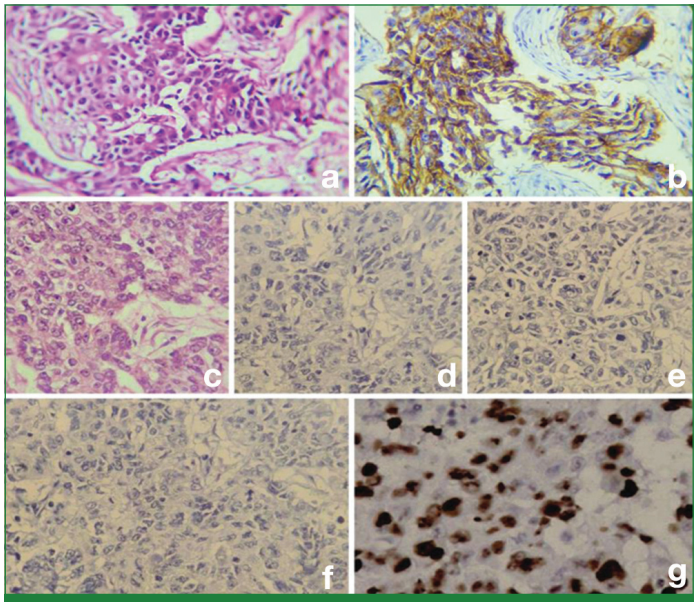
[Table/Fig-5]: Photomicrographs of a Luminal-B molecular subtype: (a-e)
a) Invasive Carcinoma Breast (H& E 40x), Immunohistochemical stains; b) ER+; c) PR+; d) HER2-; E: Ki-67 Positivity >14%.

Photomicrographs of IHC staining pattern in HER2 enriched carcinoma showing HER2 positivity (a,b) and triple negative cancer showing negative receptors and Ki-67 positivity >30% (c-f) shown in [Table/Fig-6a-f].

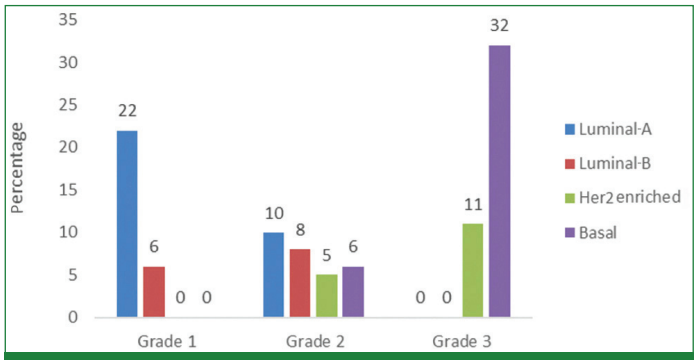
MBR histologic grades of tumours in 100 cases were grade-I (28%), grade-II (29%) and grade-III (43%). Distribution of histologic grade of 89 (100%) cases IDC-NST were: grade-I in 26 (29.2%), grade-II in 25 (28%), grade-III in 38 (42.7%). Split up of cases of Luminal-A tumours were grade-I in 68.8% and grade-II in 31.3%. Luminal-B subtype was grade-I in 42.9%, grade-II in 57.13%. There were no grade-III tumours in Luminal-A and B category. Most of HER2 enriched (68.8%) and triple negative subtypes (84.2%) were grade-III tumours, there was no grade-I tumour in HER2 enriched and triple negative category. Histologic grades and molecular types depicted in [Table/Fig-7].

Stage IA tumours (17%) in the study were Luminal-A (13%) and Luminal-B (4%). Stage-IIA tumours (19%) were Luminal-A (12%)

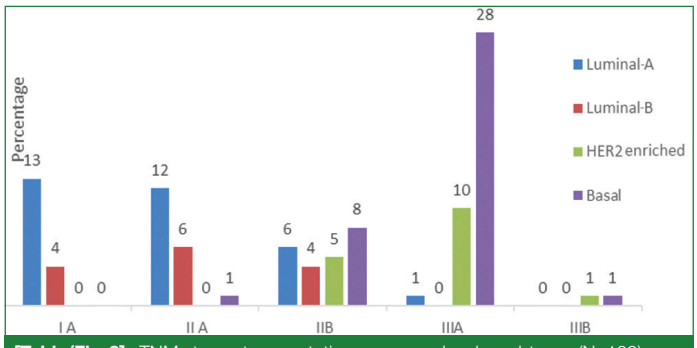
and Luminal-B (6%). In the HER2 enriched category 62.5% were in stage-3A and 6.3% in stage-3B. In the triple negative category, 73.7% tumours were stage-3A and 2.6% in stage-3B disease. In luminal subtypes, 97% of Luminal-A and 100% of Luminal-B cancers were in stage-I and II. Molecular subtypes and stage at presentation shown in [Table/Fig-8].



[Table/Fig-6]: (a,b) Photomicrographs of HER2 enriched subtype of breast cancer. a) Invasive Carcinoma Breast (H&E, 40x); b) HER2 positive; (c-g) Photomicrographs of a Triple Negative (Basal) subtype of breast cancer; c) Invasive Carcinoma Breast (H&E, 40x), immunohistochemical stains; d) ER Negative E: PR Negative; f) HER2 negative; g) Ki-67 Positivity >30%.



[Table/Fig-7]: Distribution of molecular subtypes and breast tumour Grade (N=100).



[Table/Fig-8]: TNM stage at presentation across molecular subtypes (N=100).

DISCUSSION

The present study was conducted on 100 surgical resection specimens of invasive breast carcinoma to assess the molecular and histopathological parameters. Most cases (28%) were found in the perimenopausal age group 41-50 years. Sengal AT et al., observed a peak of cancer breast in age group between 40-50 years [16]. Telli ML et al., observed that breast cancer occurs at a younger age in Asia and the postmenopausal rise in breast cancer incidence is observed in Western populations [17]. Desai SB et al., reported carcinoma in Indian women patients is a decade younger as compared to those seen in the Western population [18].

In the present study, 89 cases (89%) were IDC breast NST, tumour size were pT2 in 38% and pT3 in 33% cases. Lymph node metastasis was seen in 52 cases (52%). Kaul R et al., reported 89% cases of IDC in their study [19]. Ambroise M et al., in a study of 321 cases described majority (83.8%) of the tumours were pT2, and metastatic lymph nodes were seen in 58.19% cases [20]. Liu X et al., described molecular subtype, peritumoural vascular invasion are useful in predicting prognosis of node negative breast cancer [21].

Percentage of MBR tumour grades were, grade-I (28%), grade-II (29%) and grade-III (43%). The majority of grade-I and grade-II tumours were ER and PR positive and had low Ki-67 proliferation index. All grade-III tumours (100%) were ER and PR-negative and had a high Ki-67 proliferation index. Gogoi B et al., observed, grade-I (8.1%), grade-II (41%) and grade-III (50.4%) in their study [22]. Rakha EA et al., observed that histological grading, provides a simple and highly accurate alternative method for assessing tumour biological characteristics prognosis and identifying patients at high and low risk [23].

A comparison of biomarker testing and molecular subtypes in the present study and in other studies shown in [Table/Fig-9].

Authors, place, year	Luminal-A	Luminal-B	HER2 enriched	Triple negative
Zubeda S et al., [27] South India (2013)	19%	10%	25	46%
Liu X et al., [21] China (2014)	24%	28.8%	15.6%	31.6%
Urmila Devi P et al., [25] South India (2015)	26.8%	19.7%	12.1%	41.4%
Gogoi B et al., [22] North East India (2016)	19.5%	21.13%	17.8%	38.21%
Present study South India (2023)	32%	14%	16%	38%

[Table/Fig-9]: Comparison of proportion of molecular subtypes in other studies. (N=100).

In the present study, 38% cases were ER and PR positive, 8% were ER positive and PR negative and 54% were both markers negative. Thakur KK et al., found that the overall positivity rate for ER and PR is lower in India, as compared to Western literature. A reason could be that in Indian population, patients present in the advanced stage leading to poor outcome [24].

There were 75% HER2 negative, 23% HER2 positive and 2% equivocal tumours in our experience. The new 2018 ASCO/CAP guideline recommends the integration of IHC and ISH results, which will lead to positive or negative HER2 status with the elimination of the equivocal category [8]. Telli ML et al., in a study on breast cancer subtypes in a cohort of Asian-Americans, reported a significantly increased risk of Asians being diagnosed with HER2-positive breast cancer [17]. Urmila Devi P et al., described triple negative cancer in 54.8% of infiltrating duct cell carcinoma [25]. Inwald EC et al., described higher Ki-67 index correlates with larger tumours, positive lymph nodes, negative ERs, and positive HER2 receptors [26].

Majority (38%) of tumours were triple negative (basal like) in our experience. Zubeda S et al., in a study of breast cancer conducted in South India, described 46% triple negative tumours [27]. Triple Negative Breast Cancer (TNBC) is an aggressive and heterogeneous molecular subtype. In a meta-analysis, by Sandhu GS et al., prevalence of TNBC in India ranged from 27% to 35% across studies, with a summary estimate of 31%. They presented early in premenopausal females with large-sized lumps, high histological grade, and advanced stage at the time of diagnosis [28].

The present study showed a significant association between histological diagnosis and ER, PR, and Ki-67 positivity. High ER, PR expression was seen with low-grade-IDC, ILC, and invasive papillary carcinoma. Lower ER, PR expression and high Ki-67 labelling index more than 30% was seen with high-grade-IDC and IDC with

medullary features. As tumour size, tumour grade, and tumour stage increased, ER, and PR expression decreased and Ki-67 positivity increased (p-value <0.001). Triple negative tumours were associated with large tumour size, higher grade, higher stage, and lymph node metastasis. A significant association between molecular subtypes and histopathological parameters were seen. Sandhu GS et al., described the factors that may account for the higher prevalence of TNBC among Indian breast cancer patients are lifestyle factors such as diet and obesity, reproductive factors, genetic factors and BRCA1 (BReast CAncer gene 1) mutations [28].

Limitation(s)

Correlation with Fluorescence in situ hybridization (FISH) analysis, gene expression tests was not available in the centre.

CONCLUSION(S)

There is significant relationship observed between biomarker status and histopathological parameters. Luminal-A subtype was associated with lower histologic grade and non luminal subtypes were associated with high histologic grade tumours. Majority of breast cancers were triple negative (basal-like) in our experience, indicating a higher prevalence of aggressive subtypes of breast cancer in our community. The use of biomarkers determine prognosis and patients are benefitted with targeted therapies hence must be tested routinely. To determine molecular subtypes, IHC can be used as a surrogate for molecular testing.

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PARTICULARS OF CONTRIBUTORS:

1. Senior Resident, Department of Pathology, Government Medical College, Ernakulam, Kerala, India.
2. Professor, Department of Pathology, Government Medical College, Ernakulam, Kerala, India.
3. Additional Professor, Department of Pathology, Government Medical College, Ernakulam, Kerala, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Jayasree Raman,
Professor, Department of Pathology, Government Medical College,
Ernakulam-683503, Kerala, India.
E-mail: jayasreeraman2007@gmail.com

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