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CLASSIFICATION OF BREAST CARCINOMA BASED ON IMMUNOHISTOCHEMISTRY – AN EXPERIENCE IN A TERTIARY CARE HOSPITAL.

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ABSTRACT

Aim of the study:

The aim of this study is to analyse and catogorize breast carcinoma cases into molecular classification based on Immunohistochemistry.

Materials and Methods:

This is a retrospective study which includes 100 cases of invasive carcinoma of breast, diagnosed over the past 4 years (2013 -2016) in our centre.

These cases were classified into Luminal Type A, Luminal type B, Her 2 Neu type, Triple negative / Basal type based on Immunohistochemistry.

Results:

Analysis of the cases showed that out of 100 cases of invasive breast carcinoma, 29 were Luminal Type A, 13 were Luminal Type B, 32 were Her2 Neu type and 26 were triple negative molecular type of mammary cancer.

Introduction:

Breast carcinoma is one of the leading cause of death among women worldwide(1). In India, breast cancer is the most commonly diagnosed cancer(2).

Breast cancer is not a single disease but heterogenous disease where morphologically identical ones can display divergent clinical outcomes and response to therapy, predominantly attributed to molecular class difference amongst histologically similar cancer types(3).

In Indian females Breast cancer has been ranked as number one cancer with age adjusted rate as high as 25.8 per 100,000 women with mortality of 12.7 per 100,000 women(4).

Hence molecular classification plays an important role in predicting the prognosis and to determine the treatment modality.

In molecular classification, the breast carcinomas are divided into 4 major molecular groups: Luminal A, Luminal B, Luminal epidermal growth factor receptor 2(Her 2) and triple negative type. Molecular subtyping by immunohistochemistry is now regarded as cornerstone for detection(5). Tumour sensitivity to hormonal therapy and subsequent transtuzumab therapy determines the course of disease and prognosis.Further, racial differences in distribution of breast cancer are well documented. While luminal A is the most prevalent subtype in most regions, it is worth noting that the frequency of triple negative tumours is high among certain communities such as African Americans, Omani and Tunisians and Her 2 positive tumours among Saudi Arabians, thus highlighting the difference in pattern of distribution of molecular type among certain western population and the other populations(5).

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In this study we aim to assess the prevalence of molecular subtype of breast carcinoma in a tertiary care centre in South India. We hope that such as study will increase our understanding of breast cancer and improve its management.

Materials and methods:

This is an observational retrospective study based on data retrieved from Sri Ramachandra University (SRU). We utilized SRU's histopathology laboratory reports to identify all patients diagnosed with primary invasive breast carcinoma in the past 4 years, between January 2013 and December 2016.

We excluded male patients, recurrence cases and Non –Indian patients. Non Indian patients were excluded from this study as racial differences in incidences of carcinoma were noted in previous studies.

A total of 100 cases were selected for the study using randomisation. Using SRU's laboratory archival information systems, we obtained the following parameters for each patient: Age at diagnosis, tumour size, histopathological subtype, nottingham's histological score, presence or absence of carcinoma- in situ component, lymph node status, TNM staging, Immunohistochemical profile of the hormonal receptors ER and PR, and immunohistochemical profile of HER2 in the invasive malignant cells.

The nottingham's histologic scoring was done for each case based on the percentage of tubular/ glandular differentiations, the presence of nuclear atypical/ pleomorphism and the number of mitosis. The status of lymph node metastatic including the number of lymph node metastasis including the number of lymph nodes identified and number of lymph node positive were determined.

The immunohistochemical antibodies used for estrogen, progesterone and Her 2 are anti estrogen receptor antibody (EPR 7032), anti progesterone receptor antibody (PR 88) and anti –Her-2 (EP3) monoclonal primary antibody. Both antibodies and machines are manufactured by Biogenex, USA. The ER, PR, and HER2 were scored according to the guidelines of the college of American pathologists. Positive ER or PR requires ≥ 1% of invasive malignant cells that show nuclear staging / immnunoreactivity. The scoring for ER and PR is done as in table 1 and 2.

Table 1

Scoring criteria for ER and PR immunoreactivity

SCORE	**NUCLEAR STAINING**
0	0%
1+	< 10 %
2+	10 – 75 %
3+	>75%

The Her 2 was scored from 0 to 3, in which score 0 or 1 are negative; 2 is equivocal, 3+ is positive.

Table 2

Scoring criteria for HER 2 immunoreactivity

SCORE	**MEMBRANE STAINING**
0	Negative.No staining or membrane staining of < 10 % tumor cells
1+	Negative.A faint / barely perceptible membrane staining in >10% of tumor cells
2+	Weakly positive/equivocal.A weak to moderate ***complete*** membrane staining in > 10 % tumor cells
3+	Positive. A strong complete membrane staining in > 10 % tumor cells

Specimems showing weakly positive /equivocal Her 2 scoring were suggested to undergo flurescence in situ hybridization to determine Her 2 amplification.

The invasive breast carcinoma are classifiend into 4 molecular subtypes according to ER/ PR and Her 2 neu status as Luminal A, Luminal B, Her 2 postive and triple negative types(Table 3).

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Table 3

Molecular classification of breast carcinoma based on immunohistochemistry

SUB TYPE	ER	PR	HER 2 NEU
Luminal type A	Positive	Positive /negative	Negative
Luminal type B	Positive	Positive	Positive
Her 2 neu type	Negative	Negative	Positive
Triple negative type	Negative	Negative	Negative

Statistical analysis:

Statistical analysis was done on the data collected by using the " SPSS Version 11" statistical program. Pearsons Chi Square test was used to determine significant clinicopathological differences in expression of ER ,PR and Her 2 Neu in positive and negative tumors. Differences were considered statistically significant when p value was <0.05.

Results:

A Total of 100 breast cancer cases were included with an average patient's age at diagnosis of 49.6 years. Most cases (87%) were ductal, 12% were lobular and the remaining cases (1%) were of other subtypes including medullary, tubular, and papillary carcinoma.

The ER immunostain was positive in 42 cases and PR in 13 cases. Human epidermal growth factor receptor 2 immunostain was positive in 45 cases. The most prevalent molecular subtype was Her 2 Neu (32%) followed by, in descending order of frequency, Luminal A(29%), Triple negative(26%), and Luminal B(13%). The distribution of clinical and pathological characteristics among the various molecular subtypes is illustrated in table 4 and 5.

Table 4: The distribution of clinical and pathological characteristics among the various molecular subtypes

S.No	Characteristics	Luminal-A (%)	Luminal-B(%)	Her 2 type(%)	Triple negative(%)	Total (%)	P' value
1	Total	29	13	32	26	100	
2	Age ≤ 50 yrs	14	5	14	10	43	>0.05
	Age >50 yrs	15	8	18	16	57	
3	Tumor size ≤ 2 cm	3	1	2	4	10	>0.05
	>2 - ≤5 cm	20	8	24	16	68	
	>5 cm	6	4	6	6	22	
4	Lymph vascular invasion– Negative	17	7	12	18	54	>0.05
	- Positive	12	6	20	8	46	

Table 5 :The distribution of histopathological characters according to hormonal and molecular subtypes in 100 women with invasive breast cancer

S.No	Characteristics	Luminal A(%)	Luminal B (%)	Her 2 type(%)	Triple negative(%)	Total (%)
1	Total	29	13	32	26	100
2	Histological type- Ductal	25	10	28	24	87
	Lobular	3	3	4	2	12
	Others	1	0	0	0	1
3	Tumour grade- Grade I	1	3	2	2	8
	Grade II	22	6	12	6	46
	Grade III	6	4	18	18	46
4	Carcinoma in situ - Absent	16	2	20	22	60
	Present	13	11	12	4	40

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Triple negative tumors occurred in higher frequency in patients who were younger than 50 years of age compared with luminal tumours. Human epidermal growth factor receptor 2-positive had a tumor mass size of >2 cm in 93.75 % and triple negative tumors in 84.61% of patients ; in which most ranged between 2 cm and 5 cm (her 2 neu- 75% and triple negative – 61.53%) and the remaining were >5 cm (her 2 neu -18.75% and triple negative- 23.07%).

In addition, approximately two-thirds of these subtypes had aggressive grade 3 microscopic features . In addition, in situ component was mostly displayed in triple negative tumors (22%).

Discussion:

We studied the distribution of the molecular subtypes of breast cancer in a tertiary care hospital setting and evaluated the differences in clinico pathological features between these subtypes. The average age of diagnosis in our study was 53.35 years. Most of our cases (57%) occurred in women more than 50 years and 43% occurred in women less than 50 years.The findings are in contrast to the study by Alnegheinish et al ,where 57.9 % cases occurred in women less than 50 years and 42.1% tumors in women more than 50 years(5).

The distribution of molecular subtypes of breast carcinoma by immunohistochemistry in current study and others

Variables	Carolina USA, African Americans	Non AfricanAmericans	Saudi Arabia	Present study
No of patients	196	300	359	100
Duration	1993-96	1993-96	2010-14	2013 -16
Luminal A	47.4 %	54.0%	58.5%	29%
Luminal B	12.7%	17.3%	14.5%	13%
Her 2 neu	8.2%	5.6%	12.3%	32%
Triple neg	31.6%	23.0%	14.8%	26%

Only 10% of our patients presented with a tumour size of ≤ 2 cm, unlike USA where the percentage of patients presenting with a tumour size ≤ 2 cm is 58.4%. This signifies late diagnosis in our community,which is due to multifactorial causes such as inadequate awareness in the community regarding breast cancer and due to the presence of a non-comprehensive screening program.

Lymph vascular invasion was found in 46% of patients , among which 20 patients (43.47%) belong to Her 2 neu subtype.This is in concordance with the study by Alnegheinish et al, where lymph involvement is noted in 64.8% of patients(5).

Unlike the other western studies where luminal A is the most frequently encountered subtype, in our study Her 2 neu type is the most prevalent molecular subtype in our tertiary care centre, while luminal A is only the second most prevalent subtype(5).

Human epidermal growth factor receptor 2-positive and triple negative tumors showed a inceased frequency of grade 3 carcinomas(78.26%). This is in concordance with the study by Alnegheinish et al, where poorly differentiated carcinomas where associated with Her 2 neu and triple negative types(5).

In contrast to luminal A tumors (13%), only 4% of triple negative tumors displayed an in-situ component, which is also in concordance with the study conducted by Alnegheinish et al, where in situ component was displayed by 53.3% luminal A tumors and only 30.2 % triple negative tumors showed in situ component.

Study limitation:

Our study was limited by the absence of Ki67, a cellular marker which indicates proliferation that differentiates non-HER2 expressing luminal B from luminal A molecular subtypes. We were also limited by the absence of cytokeratin 5/6 and EGFR which helps in detecting Basal-like triple negative type of breast carcinoma.

C**onclusion**:

The molecular classification of breast carcinoma by immunohistochemistry revealed that in the patients presented in our tertiary care centre, Her 2 neu type was the most common molecular subtype, followed by luminal A tumors, which indicates the geographical variation of expression of molecular subtypes of breast cancer. Breast cancer subtypes exhibited particular characteristics.The HER2-positive and triple negative tumors were associated with an increased frequency of a large tumor size and grade 3 carcinomas and are thereby more aggressive. In addition, triple negative tumors least frequently showed a component of carcinoma in situ. We found no correlation between lymph vascular invasion and molecular subtypes.

At diagnosis, most breast cancers in our study exceeded 2 cm in maximum dimension, the cause of which needs to be examined and addressed with some urgency.

We recommend further studies on molecular subtypes of mammary carcinoma and its geographical variation for appropriate patient management.

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