RESEARCH ARTICLE

MOLECULAR CLASSIFICATION OF BREAST CANCER WITH IMMUNOHISTOCHEMICAL MARKERS

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Manuscript Info

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Abstract

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Introduction: Breast cancer is one of major cause of female mortality worldwide. The molecular classification has considered to mainly aid clinicians to better approach the prognosis and also to formulate treatments for different prognosis. According to St.Gallen symposia, five intrinsic subtypes are established: luminal A-like, luminal B-like, HER2 positive & triple negative. The aim of study is to classify carcinoma of breast into molecular classes using IHC markers in carcinoma breast.

Material & Methods: This is descriptive, cross sectional study. Histologically diagnosed cases of carcinoma of breast by H&E stain were stained by IHC markers ER, PR, HER 2/neu, ki67 & CK5/6 & interpreted. Result and Discussion-Out of 134 cases diagnosed as carcinoma breast,120 cases belongs to IDC, NST subtype. Maximum cases were from age group of 41-60 years. Grade II was most commonly seen among the total cases in present study followed by grade 3 and grade 1 respectively. HER 2 enriched provides better therapeutic options like treatment with Herceptin for HER2/neu positive cases.

Conclusion: The molecular classification using IHC surrogate markers showed more number of cases in subtype HER2(+) Enriched, luminal B, Her2(+) and triple negative.

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Introduction:-

*   Breast cancer is a heterogeneous disease, comprising numerous distinct entities that have different biological features and clinical behavior.
*   In 2018, a total of 2.1 million women were diagnosed with breast cancer, approximately one new case diagnosed every 18 seconds.[1]
*   In addition, breast cancer also represents the highest cancer mortality rates in women across the globe (103 out of 185 countries), with roughly 626,600 deaths due to the disease.

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- **with the main exceptions being the countries of Northern Europe, South America, North and Sub-Saharan Africa, where the main causes of death were due to cervical and/or lung cancer.**
- **With an age-standardized incidence rate of 25.8 cases per 100,000 women per year, it is the most common cancer among Indian women's.**
- **There is a potential risk of increase in the incidence of breast carcinoma due to adoption of western social lifestyles like delayed pregnancy, shorter duration of breastfeeding, decreasing parity.**
- Breast cancers are clonal proliferations that arise from cells with multiple genetic aberrations, which is influenced by hormonal exposures and inherited susceptibility genes.
- Approximately 12% of breast cancers occur due to inheritance of identifiable susceptibility genes.
- Mutations in BRCA1 and BRCA2 are responsible for 80% to 90% of single gene familial breast cancers and about 3% of all breast cancers.
- **BRCA1 (on chromosome 17q21) and BRCA2 (on chromosome 13q12.3) are large genes and hundreds of different mutations distributed throughout their coding have been associated with familial breast carcinoma.** The extent of the heterogeneity of breast cancer was highlighted by the publication of microarray-based studies that identified multiple molecular subgroups.[2]
- 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.[3,4]
- Lack of widespread use of expression profiles is primarily due to the expense and technical difficulty encountered when carrying out high-throughput gene- expression profiling using paraffin-embedded material.
- Moreover, the currently defined subtypes based on expression profiling were determined through the study of relatively small numbers of tumors and these subgroups may not be definitive.
- Consequently, there is interest in using immunohistochemical (IHC) markers to classify tumors into subtypes that are surrogates for those based on gene-expression profiling.
- Different molecular subtypes of breast cancer reveal certain specific characteristics and thus was thought that specific and targeted therapy could be developed.
- In order to assign the tumors into various subtypes, efforts have been made to use immunohistochemistry as surrogate (antibodies like ER, PR, HER2, cytokeratin 5/6, EGFR, Ki-67).
- Clinical uptake of the surrogate classification of breast cancers by 4-IHC is also largely attributable to the St Gallen symposia of 2011 and 2013, at which five intrinsic subtypes were established: luminal A-like, luminal B-like (HER2 negative); luminal B- like (HER2 positive); HER2 positive (non-luminal), and triple negative (ductal).
- Immunohistochemical determination of ER, PR, HER2neu levels became known as 4- IHC with incorporation of ki67 expression levels as a measure of proliferative rate into routine evaluation of invasive breast carcinoma.
- **Oncologists treating patients with luminal-type breast cancers, that is, those with hormone receptor positive tumors, may opt for one of the genomic prognostic tests to determine the need for adjuvant chemotherapy.[4]**

Aims and Objectives:-
Aim:-
To classify breast carcinoma into molecular classes using immunohistochemical markers   in breast carcinoma.

Objectives:-
- To classify breast carcinoma into molecular classes using immunohistochemical markers.
- To study and evaluate the morphological patterns of carcinoma breast with molecular class.
- To study the age distribution of carcinoma breast.
- To study the staging and grading of carcinoma breast with molecular class

Materials and Methods:-
- Study type : Observational Study(Descriptive cross-sectional study.)
- Study Duration :   January 2022 to June 2023.
- Setting :   Department of Pathology, Loni

Inclusion criteria :
All histopathological cases diagnosed as Invasive breast carcinoma on HPE slides.

Exclusion criteria :
- Men with breast carcinoma.
- If blocks are not available.
- If the blocks are exhausted.

Methodology:-

A retrosprective study of breast specimens were taken.

Morphological type	28-40 yrs	41-50 yrs	51-60 yrs	61-70 yrs	71-80 yrs	Total
IDC,NST	13	38	36	22	11	120
ILC	0	0	0	4	0	4
Lymphoma	0	2	0	0	0	2
Medullary carcinoma	0	6	2	0	0	8
Total	13	**46**	38	26	11	134

2. A detailed history of every patient and clinical features obtained from the records.

3. The section were stained for ER,PR,HER2/Neu ,ki67 and CK5/6.
4. Sections are classified in molecular classification.

Result and Discussion:-

Age distribution among morphological subtypes:

Comparison of molecular subtypes of carcinoma breast:

	Total	IDC,NST	ILC	Lymphoma	Medullary carcinoma	Others.
Present study	134	120	4	2	8	0
KaranganaEt al	60	54	1	0	4	1
Mittal et al	70	47	-	_	_	_
Ansari et al	516	496	12	_	2	6

Comparison laterality of carcinoma breast :

Study	Left	Right
Present study	57.5%	42.5%
Mittal et al	69%	31%
Geethamala et al	50%	49%

Comparison of ER positivity, PR positivity and HER2/neu positivity in carcinoma breast :

Study	ER +VE	PR +VE	Her2(+)
Present study	**49.17%**	**40%**	**64%**
Ankit Mittal et al	52%	43%	**45%**
Geetamala et al	55%	**53%**	**25%**
Ansari et al	57.5%	44.1%	**26.6%**
Karangadan et al	45%	41.67%	61.67%

Comparison of ER positivity, PR positivity and HER2/neu positivity in carcinoma breast :

Variabls		Number
Luminal A		**10 (8.33)**
Luminal B		**50 (43.33)**
	HER2-	20 (16.66)
	HER2+	30 (25.00)
HER2(+) Enriched		**40 (32.33)**
Triple Negative		**20 (16.33)**
	Negative	15 (12.5)
	Basal type	5 (4.55)

Classification of molecular subtypes of breast carcinoma:

Study	ER +VE	PR +VE	Her2(+)
Present study	**49.17%**	**40%**	**64%**
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Ansari et al	57.5%	44.1%	**26.6%**
Karangadan et al	45%	41.67%	61.67%

Comparison of molecular subtypes in carcinoma breast:

Study	Luminal A	Luminal B	Her2Neu	Triple negative
Present Study	**8.33%**	**43.33%**	**32.33%**	**16.33%**
Shukla et al	28.07%	29.82%	26.31%	15.78%

Ansari et al	41.7%	15%	12.5%	30.8%
Ankit Mittal et al	22%	28%	25%	25%
Walke et al	21%	8%	19%	51%
Setyawati et al	41.3%	13.8%	19.4%	25.5%
Cheang et al	46.5%	17%	15%	21.5%

Molecular subtypes according to grading:

Variable		No.of cases	Grade 1	Grade 2	Grade 3
Luminal A		10	0	8	2
Luminal B		50	9	32	9
	HER2(-)	20	0	16	4
	HER2(+)	30	4	21	5
HER2+		40	0	31	9
TN		20	2	12	6
	Negative	15	2	11	3
	Basal type	5	0	3	2

Comparison of Grade in carcinoma of breast:

Study	Grade 1	Grade 2	Grade 3
Present study	**8.6%**	**67.5%**	24.3%
Ansari et al	28.7%	56.8%	14.5%
Shukla et al	22.8%	36%	41.2%
Walke et al	40.4%	31.9%	27.6%

Molecular subtypes according to staging:

Variable		Number	T1	T2	T3	T4
Luminal A		10	1	5	1	0
Luminal B		50	5	5	5	0

	HER2(-)	20	3	4	0	0
	HER2(+)	30	2	18	0	0
HER2(+)		40	4	15	4	1
TN	Negative	20	4	6	0	1
	Basal type	15	3	2	0	1
		5	1	4	0	0

Comparison of staging in carcinoma breast :

Study	T1	T2	T3	T4
Present Study	20.22%	64.20%	14.25%	2.50%
Mittal et al	9%	56%	34%	0%
Setyawati et al	17%	44.5%	38.4%	0%
Gethamala et al	28%	57%	15%	0%
Walke et al	10.6%	61.7%	27.6%	0%
Shukla et al	2.6%	61.4%	35.9%	0%

Summary:-

1. Out of total 134 total cases ,120 cases belongs to IDC,NST subtype.
2. Carcinoma breast **IDC,NST subtype is most common morphological subtypes.**
3. **Maximum subject groups were from age 41-60 years (60%) followed by 61-70 years (20%).**east is most commonly in 4th to 6th decade.
4. Most women had cancer of left breast (57.5%) **as compared to right side.**
5. ER positive outcome was found in 59 cases (49.17%),PR positive outcome was seen in 48 cases (40%) and Her2/neu outcomes was seen in 77 cases (64.1%).
6. According to molecular classification maximum 50 cases were found in Her2(+) Enriched followed by Luminal B subtype which included 30 cases.
7. Grade 2 tumors were the most common and among them Her2(+) Enriched was most common 32 cases.
8. T2 stage tumors were the most common in 59 cases and among them Her2(+) Enriched was most common 18 cases.
9. IDC,NST subtype was the most common morphological subtype and among them Her2(+) Enriched was the most common molecular subtype.

Conclusion:-

1. The molecular classification using IHC surrogate markers showed more number of cases in subtype HER2(+) Enriched, Luminal B, Her 2(+) and Triple negative.
2. This implies poor prognosis of cases but provide better therapeutic option like treatment with Herceptin for HER2/neu positive cases
3. Prolong the survival among patients of breast carcinoma were studied IDC ,NST was commonest reported 4th and 6th decade.
4. Present study maximum cases were Grade 2 and stage T2 tumors implying overall intermediate prognosis.