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RESEARCH ARTICLE

TRIPLE MARKERS (ER, PR, HER 2 NEU) AND THEIR RELATIONSHIP WITH KI67 EXPRESSION IN BREAST CARCINOMA - AN OBSERVATIONAL STUDYIN NORTH EAST INDIA

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Abstract

Background: Breast cancer is the most common and deadly malignancy of women globally. Breast cancer is called ER/PR positive if it has receptors for hormone estrogen/progesterone. Similarly HER2neu positive status of breast carcinoma means that HER2neu gene is making too many HER2neu proteins. Hormone receptor studies with ER, PR & HER2neu are routinely done in breast carcinoma. Ki67 is an independent factor to predict tumour grade. Breast carcinoma can therefore be classified based on expression patterns of ER, PR, HER2neu & correlated with Ki67 into various molecular subtypes.

Objectives:(1) To determine the association between expression patterns of ER, PR and HER2neu with Ki67 in breast carcinoma in patients attending our hospital. (2) To estimate the proportion of different molecular subtypes of breast carcinoma.

Materials & Methods: It was an observational cross sectional study done on 122 cases for a period of 2 years(July 2020- June 2022) and surgical specimens from patients operated for breast carcinoma of all ages along with true cut biopsies were included. Immunohistochemical study for ER, PR, HER2Neu & Ki67 were performed on breast tissue specimens received.

Results: The study found inverse correlation of Ki67 with ER, PR and its direct correlation with Her2Neu. TNBC (Triple Negative Breast Carcinoma) was the most predominant molecular subtype.

Conclusion: High Ki67 is considered as an unfavourable factor influencing tumour progression and is associated with poor prognosis. Ki67 is a potential prognostic biomarker in breast cancers and such prognostic information could be beneficial for development of therapeutic strategy.

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Introduction:-

Breast cancer is the most common and deadly malignancy of women globally; each year 1.7 million women are diagnosed and one in three of those affected die of this disease. It accounts for one third of all malignancies affecting women.(1)Breast cancer is called ER/PR positive if it has receptors for hormone estrogen/progesterone, which receives the signals from estrogen & progesterone and promotes its growth, just like normal cells. Similarly HER2 neu positive status of breast carcinoma means that HER2 neu gene is making too many HER2 neu proteins. Hormone receptor studies such as ER, PR & HER2 neu are routinely done in breast carcinoma. It not only helps in assessing prognosis of tumour but also helps in deciding its treatment. The presence of the hormone receptors ER/PR in a patient's breast cancer is an example of weak prognostic but strong predictive biomarker. HER2 neu overexpression is considered to be both prognostic as well as predictive.

Ki67 is an independent factor to predict tumour grade. A higher ki67 index correlates with poor prognosis & early recurrence. Breast carcinoma can therefore be classified based on expression patterns of ER, PR, HER2 neu & correlated with Ki 67. Molecular classification of breast cancer along with Ki 67 index is considered a better predictive factor for prognosis and treatment than routine histopathology.

The study was conducted as it would help in planning the prognostic and therapeutic approach in patients with breast carcinoma as no such study has been done from the state of Tripura, available in literature.

Aim:-

To assess triple markers (i.e ER, PR, HER2 neu) in breast carcinoma and to analyse their association with Ki67 expression.

Objectives:-

1) To determine the association between expression patterns of ER, PR and HER2 neu with Ki67 in breast carcinoma in patients attending our hospital.
2) To estimate the proportion of different molecular subtypes of breast carcinoma namely Luminal A, Luminal B, Triple negative, HER2 enriched in aforesaid study population.

Materials & Methods:-

It was an observational cross sectional study done on 122 cases for a period of 2 years(July 2020- June 2022) and surgical specimens from patients operated for breast carcinoma of all ages along with tru-cut biopsies were included while cases with extensive tumour necrosis without sufficient viable tumour cells and those who had received neoadjuvant chemotherapy were excluded. Immunohistochemical study for ER, PR, HER2Neu & Ki67 were performed on breast tissue specimen received after mastectomy/lumpectomy/tru-cut biopsy.

The technique for IHC included antigen retrieval in tris buffer, blocking endogenous peroxidase using hydrogen peroxide, incubating with primary monoclonal antibody, developing chromogen with diamino-benzidine (DAB) & counterstaining with haematoxylin. The immunostained slides had been examined for nuclear staining in case of ER, PR and Ki67 &cytoplasmic membrane staining in case of HER2 neu.

Results:-

ER status	Ki67 expression-High		Ki67 expression-Low		Total		P value
	n	%	n	%	n	%	
Positive	11	23.9	35	76.1	46	100	
Negative	76	100	0	0	76	100	<0.001
Total	87	71.3	35	28.7	122	100	
Chi square value=81.09							

Table 1:- Correlation of ER and Ki67 expression status.

The study showed 11 ER positive and 76 ER negative cases showing high Ki67(>20%). There were 35 ER positive and no ER negative cases showing low Ki67(<20%) . It is evident from the result that the ER positive cases had low Ki67 and ER negative cases were having high Ki 67.

PR status	Ki67 expression-High		Ki67 expression-Low		Total		P value
	n	%	n	%	n	%	
Positive	7	16.7	35	83.3	42	100	
Negative	80	100	0	0	80	100	<0.001
Total	87	71.3	35	28.7	122	100	
Chi square value=93.49							

Table 2:- Correlation of PR and Ki67 expression status.

The study showed 7 PR positive and 80 PR negative cases showing high Ki67(>20%). There were 35 PR positive and no PR negative cases showing low Ki67(<20%) . It is evident from the result that the PR positive cases had low Ki67 and PR negative cases were having high Ki 67.

Her2Neu status	Ki67 expression-High		Ki67 expression-Low		Total		P value
	n	%	n	%	n	%	
Positive	25	100	0	0	25	100	
Negative	59	64.1	33	35.9	92	100	0.002
Equivocal	3	60	2	40	5	100	
Total	87	71.3	35	28.7	122	100	
Chi square value=12.69							

Table 3:- Correlation of Her2Neu status and Ki67 expression status.

The study showed 25 Her 2 neu positive, 59 Her 2 neu negative cases and 3 Her 2 neu equivocal cases showing high Ki67 (>20%). There were no Her 2 neu positive, 33 Her 2 neu negative cases and 2 equivocal cases which were showing low Ki67 (<20%). It is evident from the results that the Her 2 neu positive cases had high Ki67 index. Her 2 neu negative cases and Her 2 neu equivocal cases were also having high Ki 67 index.

Molecular diagnosis	Number	Percentage(%)
HER2 Enriched	24	19.7
Luminal A	35	28.7
Luminal B	11	9.0
TNBC	52	42.6
Total	122	100.0

Table 4:- Molecular diagnosis.

All the cases of breast carcinoma were divided into 4 molecular subtypes namely Luminal A, Luminal B, Her 2 Enriched and Triple Negative Breast Cancer (TNBC). The molecular subtype having the highest proportion was TNBC followed by Luminal A, Her 2 enriched and Luminal B.

Molecular diagnosis	Ki67 expression-High		Ki67 expression-Low		Total		P value
	n	%	n	%	n	%	
TNBC	52	100.0	0	0.0	52	100.0	
Her2 enriched	24	100.0	0	0.0	24	100.0	
Luminal B	11	100.0	0	0.0	11	100.0	<0.001
Luminal A	0	0.0	35	100.0	35	100.0	
Total	87	71.3	35	28.7	122	100.0	
Chi square value=122.0							

Table 5:- Molecular diagnosis V/S Ki67 expression status.

The study showed that all the TNBC, Her 2 enriched, Luminal B cases were associated with high Ki 67. In contrast, all the Luminal A cases were associated with low Ki67.

Discussion:-

Breast cancer is the most common female cancer worldwide and the second leading cause of cancer-related death, thus it is important to find good prognostic markers that can define patients who are at high risk of recurrence and choosing the suitable therapy for them. (2) Prognostic markers such tumor size, grade, age, histological type and estrogen receptor status influence the therapy decision. Cell proliferation is one of the most essential characteristics of cancer; thus, its measurement may provide useful information about disease status.(3)

In our study, all the cases of breast carcinoma had being divided broadly into two categories (<50 years and >=50 years). Cases who were aged < 50 years were the major category constituting 54.1%. In 2019, Madhushankar L et al conducted a study where most of their cases belonged to younger age group and they concluded from the study that younger the age group more aggressive is the breast cancer.(4) Similar findings were observed in the study conducted by other authors where they showed strong association between TNBC subtype and age < 50 years.(5,6)These findings are similar to findings of our study.

In our study estrogen receptor (ER) & Progesterone receptor(PR) had an inverse relationship with Ki67. Considering each biomarker individually, results of Ahmed ST et al 2018, Sheikhpour R et al 2016 and Nishimura R et al 2011 also showed a significant inverse relationship of Ki67 with ER and PR in contrast to the direct correlation with Her2/neu status. (7)

The significant direct relationship between Ki67 expression and HER2/neu positivity among our study series is similar to what have been reported by many authors who detected a higher correlation between HER2/neu and increased expression of Ki67 among women with breast cancer.[7,8,9]

In our study, higher Ki-67 expression was more frequently associated with HER2-negative. This result was in agreement with study done by Stathopoulos et al, who reported HER2-negative had significantly higher Ki-67 values.(10,11) In our study, Her2neu positive & equivocal cases had high Ki67index. In the study conduced by Park S et al (12), similar findings were observed where Ki-67 index is highly expressed in all three ER, PR and HER2 negative status. Contrary to our result, other studies founded that a higher Ki-67 index significantly correlated with HER2-positive.(13,14,15)

Even though Ki-67 index is commonly used worldwide, standard cut-off value for Ki-67 index is controversial. Denkert et al. and Tan et al. suggested that 20% cut off value of Ki-67 impact the poor prognosis and chemotherapy treatment in breast cancer.(16) Other authors also found that 20% cut off value is more practical to apply in clinical decision making.(12) In our study, we also support that 20% Ki-67 cut off value is reliable to predict prognosis of breast cancer.

The molecular subtype having the highest proportion in our study was TNBC followed by Luminal A, Her 2 enriched and Luminal B. In a study conducted by Arpita J et al in the year 2022, TNBC was the most predominant subtype followed by Luminal A, luminal B, Her 2 enriched.(17) Our findings are concordant with this study and other studies conducted by different authors.(18,19)

Conclusion:-

The molecular classification of breast cancer by IHC in our study population showed predominance of Triple Negative Breast Carcinoma (TNBC) followed by Luminal A, Her 2 enriched and Luminal B. Our findings are concordant with other studies of North-east India where TNBC is the predominant molecular subtype in contrast to the predominance of Luminal-A in majority of studies all over India. Molecular techniques have improved our understanding of breast cancer biology. Immunohistochemistry can be used as a surrogate for molecular testing to determine the molecular subtypes. The application of these markers in clinical setting determine prognosis and have the potential to benefit patients with targeted therapies.

Our study showed inverse correlation of Ki67 with ER, PR whereas it was directly proportional to Her 2 Neu. This finding was concordant with other studies all over India. High index labelled Ki67 is considered as an unfavourable factor that influences tumour progression and is associated with poorer prognosis. It helps in counselling the patient about prognosis of the disease. Ki67 has great potential as prognostic biomarker in aggressive breast cancers and such prognostic information could be beneficial for development of therapeutic strategy. We currently still mostly

rely on manual analysis of immunohistochemistry for the evaluation of Ki67 in clinical practice. Improved reproducibility may be achieved through future approaches in digital pathology. Future focus should be on standardization of Ki67 assesment to avoid any contradictory results in Ki67.

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Conflict Of Interest:-

Nil.

Pictures :-

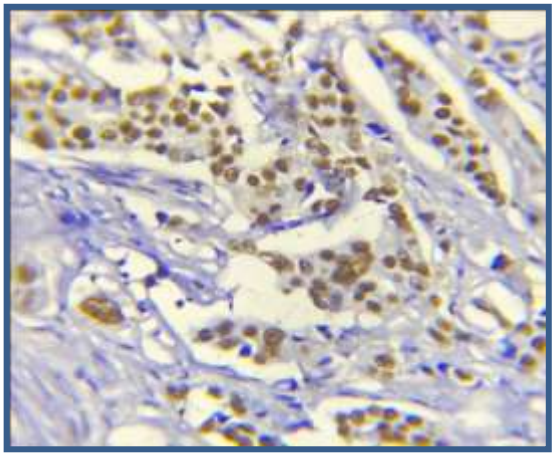

Figure 1:- ER positive (brown stained nuclei).

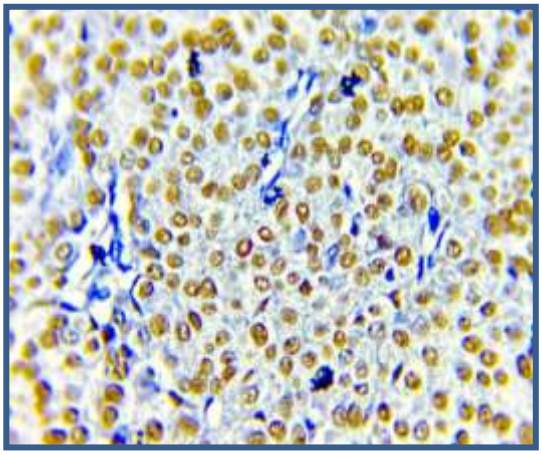

Figure 2:- PR positive (brown stained nuclei).

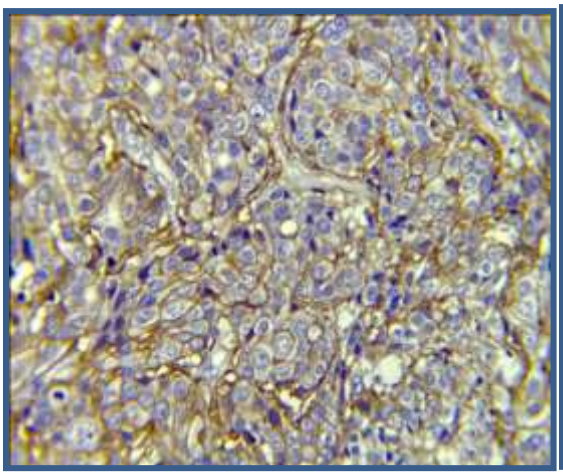

Figure 3:- HER2 neu membrane positivity.

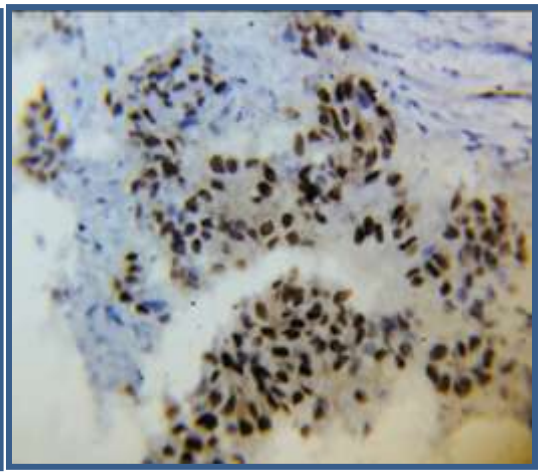

Figure 4:- High Ki67(>20%) in nuclei.

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