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Study of Molecular Markers of Breast Carcinoma and Correlation with Other Prognostic Parametres

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***Abstract**: Breast cancer is the second most leading cause of cancer mortality in Asian countries. The prognosis depends on variable clinical and tumor related factors. Assessment of molecular markers in breast cancer i.e. estogen, progesterone receptors and Her2 neu is strongly recommended to provide best therapeutic options. The aim of this study is to determine the correlation present between molecular markers and other prognostic factors in carcinoma breast. A total 100 cases of invasive duct cancers were included in this study. The hormone receptors, Her2/neu were assessed immunohistochemically and compared with age, size, grade and lymphnode status of the tumor. The prevalence of ER PR was found to be 64% and 81% of cases. Correlating the above factors with hormone receptor status, it was found there is increase positivity of ER/PR in post menopausal age group (68.2%), small tumor size(77.4%), negative lymph node status(45.16%) and moderately differentiated tumors(77.4%). Her2 was overexpressed in 24% of cases. Majority (75%) of young age group(30-39 yrs) patients showed strong positivity for Her-2/neu. Likewise it was found to be overexpressed mostly in large tumor size (40% of T3 stage & 100% of T4 stage),high grade tumors(75% of grade III tumors).Among the molecular markers the hormone receptors and the her 2neu were shown to possess an inverse relationship to each other. The prevalence of ER, PR and Her2/neu amplification in breast cancer in the present study is similar to international data and correlated significantly with other prognostic factors.*

***Keywords:** Breast Cancer, Estrogen, Her2/neu, Progesterone*

I. Introduction

Carcinoma breast is the second most common malignant tumor and the cancer related death in females.[1]It was found that the survival and mortality in breast cancer depends directly on with similar clinicopathologic prognostic factors like age, tumor size, histologic grade, nodal metastases, mitosis and necrosis[2]. Since mid 1990s the use of predictive molecular markers in breast cancer has revolutionised the approach to management and prognosis[3].The basic molecular markers that are routinely used are the estrogen and progesterone receptors, and the epidermal growth factor receptor which are newer prognostic factors and predicts the response to therapy. Basing on above three molecular markers breast carcinoma can be divided into four subtypes (IHC Classification)[4] as Luminal A, Luminal B, Her2/neu overexpressing and Triple negative .This molecular classification is the most acceptable criteria for predicting the prognosis, response to hormonal treatment, and the potential use of newer drugs such as Trastuzumab in the case of Her-2 over expression type.

Approximately 50 to70 percent of breast cancer patients have been found to contain estrogen and progesterone receptor(ER and PR)**.** Several studies have indicated that ER PR positive tumors have a better survival and favourable host-tumor relationship[5].Her2/neu is a proto-oncogene that is amplified in 15 to 30 percent of breast cancer. It predicts the resistance or sensitivity to tamoxifen or chemotherapy. But shows better responsiveness to targeted therapy.Her2/neu found to be associated with increased disease recurrence, systemic metastasis and shortened survival[6]**.**

II. Material & Method

The present study was conducted in the Department of pathology, MIMS Medical College,vizianagaram from September'2012 to September'2015. Histopathological examination of a total of 100 mastectomy specimens of patients clinically diagnosed as breast carcinoma, were conducted by using conventional H&E stain. Immunhistochemical evaluation of ER, PR & Her-2 were done on formalin fixed paraffin embedded tissue sections by using Novocastra's ready to use mouse monoclonal antibody & Novolink polymer Detection system.

Expression of these target antibodies (ER,PR & Her-2) were compared with other prognostic parameters like patient's age, menopausal status, size of the tumor, histological type , grade (MODIFIED BLOOM RICHARDSON GRADING) & lymph node status. IHC scoring was done by **H score & DAKO score** for ER/PR & Her 2 neu respectively[7].

- **H score(ER/PR)-**Summation of **proportion & intensity** of staining

- <50 -- Negative
- 51-100– Weakly Positive
- 101-200- Moderately Positive
- 201-300– Strongly Positive

(**Reactivity**: 0-no ,1-weak,2-moderate,3-strong.Maximum total score is 300 if 100% tumor cells give strong reactivity.)

- **DAKO score(Her 2neu)**
- 0(Negative)-no staining or staining of<10% of tumor cells.
- 1(Negative)- faint or partial membrane stain in >10%tumor cells.
  2(Weakly positive)-weak to moderate complete membrane staining in >10% or strong complete membrane staining in <30% of cells.
- 3(Strongly positive)-strong complete membrane staining in >30% of cells.

III. Observation

Among the 100 cases included in this study Infiltrating duct carcinoma (IDC NOS) was the largest group, accounting for 86%. Apart from IDC a single case, each from ductal carcinoma in situ(DCIS), a Lobular carcinoma in situ (LCIS),a Medullary carcinoma, a Metaplastic carcinoma, a Mucinous carcinoma was found. Among the study group,the common age group affected to be post menopausal 40-59 yrs i.e 78%(n=78). Majority of the patients were ER PR+ve 62%, 20% were ER-vePR+ve, 12% were ER PR-ve and only 6% were ER+vePR-ve. Among the total ER PR positive cases 45% belonged to 40-49 years age group, similarly among ER PR negative patients 50% were of 40-49 years of age. Comparing the ER PR status with tumor size (table 1) at presentation, it was observed that 77.41% of ER PR +ve cases were T2 stage, 16.12% were T1 and only 6.4% were at T3 stage. Whereas in ER PR –ve group 66.66% were at stage T2 and 16.66% each in T2 and T3 respectively, while there was none in T1 stage.

Correlating the nodal status and hormone receptors (table 2), we found 45.16% patients of ER PR +ve group were without any lymphnode involvement whereas ER PR -ve group 99% of patients had nodal involvement in the form of either N1 or N2. Out of 31 ER PR +ve tumors 24 (77.4%) cases are moderately differentiated (grade II) and 6.4% belong to grade III whereas 16.6% ER PR –ve cases were poorly differentiated (grade III)(Table 3).

In the present study Her2/neu was found to be positive in 24% of cases. The overexpression of Her2/neu in young age group (30-39 yrs) was found to be 75%, whereas 90% of 50-59 yrs and 86% of above 60 yrs were associated with Her2/neu negativity. Our study observed 75% patients with N_0 stage had Her/neu negative status. Similar result was found in patients with nodal involvement that is 69% and 75% of N_1 & N_2 stage showed Her2 negativity respectively.

Present study(**Table 4**) found the patients in T_1 stage ,100% cases belonged to Her2 negative status, while the patients with T_3 & T_4 stage,60% and 100% cases showed Her2/neu positivity respectively. Correlating the Her2 status with histological grading(**Table 5**), 100% grade I tumors had Her2 negative status but moving on to the higher grade,75% of grade III tumors were associated with Her2 positivity. Correlating the Her2/neu with hormone receptor status(**Table 6**), present study found that 85% ER +ve patients showed Her2 negativity,56% ER-ve tumors showed Her2 positivity but only 15% ER +ve patients had Her2 positive status. Similar result was found with progesterone receptor i.e.89% PR –ve tumors showed Her2 positivity whereas only 15% PR+ve tumors had Her2/neu positive status.

Table 1: Relationship between ER PR Receptor Status and Tumor Size in Breast Cancer Patients

		ER /PR												**Tumor Size**													**Total**			
						T_1				**T_2**				**T_3**				**T_4**							**N**			

Status	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**
ER+ve	10	16.1	48	77.4	4	6.4	0	0	62
PR+ve									
ER+ve	0	0	6	100	0	0	0	0	6
PR-ve									
ER-ve	2	10	14	70	4	20	0	0	20
PR+ve	.								.
ER-ve	0 .	0	8	66.7	2	16.7	2	16.7	12
PR-ve									
Total	**12**	**12**	**76**	**76**	**10**	**10**	**2**	**2**	**100**

Table 2: Relationship between ER PR Status and Lymph Node Involvement in Breast Cancer Patients

ER/PR Status	**LYMPH NODE**								**Total**
	N_0		**N_1**		**N_2**		**N_3**		**No.**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**
ER+ve	28	45.2	32	51.6	2	3.22	0	0	62
PR+ve									
ER+ve	0	0	4	66.7	2	33.3	0	0	6

	PR-ve															
	ER-ve	12		60	6		30	2		10	0		0	20		
	PR+ve															
	ER-ve	0		0	10		83.3	2		16.7	0		0	12		
	PR-ve															
	Total	**40**		**40**	**52**		**52**	**8**		**8**	**0**		**0**	**100**		

Table 3: Relationship between Histopathological Grading and ER PR Status in Breast Cancer Patients

S. No.	**ER PR Status**	**Histopathological Grade**						**Total No.**	
		Grade – I		**Grade – II**		**Grade – III**			
		No.	**%**	**No.**	**%**	**No.**	**%**		
1	ER+ve PR+ve	10	16.1	24	77.4	4	6.4	62	
2	ER+ve PR-ve	0	0	2	66.7	2	33.33	6	
3	ER-ve PR+ve	0	0	10	100	0	0	20	
4	ER-ve PR-ve	0	0	5	83.3	2	16.66	12	
Total		**10**	**10**	**42**	**42**	**8**	**8**	**100**	

Table 4: **Correlation of Tumor Size with Her 2/neu Status**

Tumor Size	**Her2/neu +ve**	**%**	**Her2/neu -ve**	**%**	**Total**
T_1	0	0	12	100	12
T_2	20	26	56	74	76
T3	6	60	4	40	10
T_4	2	100	0	0	2
Total	**28**	**28**	**72**	**72**	**100**

Table 5: Correlation of Her2 Staus with Istological Grading

Histological Grade	**Her2/neu +ve**	**%**	**Her2/neu - ve**	**%**	**Total**
G1	0	0	10	100	10
G2	22	27	60	73	82
G3	6	75	2	25	8
Total	**28**	**28**	**72**	**72**	**100**

Table 6: Comparison of Oestrogen and Progesterone Receptor with Her2 Status

	ER Status	**Her2/Neu +Ve**	**%**	**Her2/Neu -Ve**	**%**	**Total**

	ER –ve	18	56	14	44		32	
	ER +ve	10	15	58	85		68	
	Total	28	28	72	72		100	
	PR -ve	16	89	2	11		18	
	PR +ve	12	15	70	85		82	
	Total	**28**	**28**	**72**	**72**		**100**	

IV. Discussion

Prognostic and predictive factors are universally utilized in the management of breast cancer . ER/PR status and Her 2 Neu overexpression are both prognostic and predictive factors. Generally, ER and PR positivity and Her 2 Neu gene negativity are associated with better prognosis and vice versa.

The present study was carried out to determine the frequency of ER, PR, and Her 2 Neu positivity and correlation with menopausal status and other pathological parameters, as well as their effect on overall survival . The results obtained were found to be more or less similar to those reported in the international literature with some exceptions.

Our study found 45% of ERPR+ve and 50% of ERPR-ve cases belonged to 40-49 yrs of age so the correlation is statistically insignificant. That is consistent with barnes et al[10]. Regarding menopausal status it was observed that majority i.e.68% of postmenopausal group have ER+ve status which is similar to Hawkins et al 11 who found 61% ER positivity in the respective group. Barnes etal 1993 **10** revealed that ER+ ve tumours were smaller than ER-ve tumour, while Allegra etal 197812 found no correlation between hormone receptor positivity and size of the tumour.Our study is consistent with Barnes etal10, that 77.41% ER PR+ ve tumors were at T2 stage, whereas in ER PR –ve group 16.66% each presented with T2 and T3 stage respectively**. Allegra etal12** in their study showed that ER positive group patients had a high proportion of node negative patients. While Fatima etal13 found no significant correlation between ER PR status and lymph node metastasis. Our study is consistent with Allegra etal12 which showed that 45.16% ER PR+ve group were without any lymph node involvement whereas in ER PR-ve group 100% of the patients had nodal involvement in the form of either N1 or N2.

Among the present study group 82% of the patients had moderately differentiated(grade II) tumor, 10 % had grade I(well differentiated) tumor and the rest 8 % of the patients belonged to grade III i.e. poorly differentiated which is consistent with Azizun etal who found 55.3% moderately differentiated tumors Present study revealed that majority(77.4%) of ER PR +ve are moderately differentiated whereas only 6.4% belong to grade III. Constratly only 16.6% ER PR-ve cases were poorely differentiated. Barnes etal10 and Ratnatunga etal14 in their study on relationship between hormone receptor status and ductal carcinoma concluded that ER positivity decreases significantly for high grade tumour. Barnes etal55 in their study showed that 73% of ductal carcinoma were ER +ve ,61% were PR+ve, which is compared with present study with 62% of ERPR positivity.

In the present study Her2/neu was overexpressed in 24% of cases which is comparable with the finding of Azizun etal **8** who found Her2 positivity in 24.7% cases. The overexpression of Her2/neu was found to be more prevalent in young age i.e. 75% of 30-39 yrs patients found to be Her2 positive , whereas 90% patients of 50-59 yrs of age group and 86% patients of above 60yrs were associated with Her2/neu negativity.Kamil etal 9

did not find any association between age at diagnosis and Her2 status.In comparison to above study Azizun etal 8 reported that Her2/neu positivity decreased with advanced age which is consistent with present study.

Our study observed no significant correlation between Her2neu expression and lymphnode metastasis. 75% of N0 stage, 69% and 75% of N1 & N2 stage showed Her2 negativity respectively.Kamil etal 9 and Ivkovic etal15 also did not find any correlation between Her2 and lymphnode infiltration.But Azizun etal 8 reported Her2/neu to be positively associated with lymphnode metastasis.Present study found that in case of patients with small tumor size(T1 stage) ,100% cases belonged to Her2 negative status. On the other side as the tumor size goes on increasing that is the patients with T3 & T4 stage, 60% and 100% cases showed Her2/neu positivity respectively. The result is consistent with that of Ivkovic etal 15 and Azizun etal **8** who found Her2/neu to be strongly associated with large tumor size. Correlating the Her2 status with histological grading, present study noticed that Her2 positivity is more prevalent in higher grade tumors. All the grade I tumors had Her2 negative status but moving on to the higher grade,75% of grade III tumors were associated with Her2 positivity. This finding is comparable with that of Ivkovic etal[15]. Correlating the Her2/neu with hormone receptor status, present study found that 85% of estrogen and progesterone receptor positive tumors showed Her2 negativity.Whereas 56% ER-ve tumors showed Her2 positivity.The above findings are very much consistent with that of Ivkovic etal[15] who found 89% of ER+ve cases and 91% of PR+ve cases to be associated with Her2 negative status.Similar results were also reported by Azizun etal 8 .The above data concluding an inverse correlation between Her2neu and hormone receptors.

The present study categorised the patients according molecular markers i.e. the IHC classification**(Table 7**) which includes four Luminal A type(ERPR+ve,Her2-ve), Luminal Btype(ERPR+ve,Her2+ve), Her2 Overexpressing(ERPR-ve,Her2+ve) and Triple negative or Basal type (ERPR-ve,Her2- ve).For convenience of the categorization,present study has excluded the ER+ve/PR-ve and ER-ve/PR+ve patients.

Table 7: IHC Classification

IHC SUBTYPE	**No.**	**%**	**Follow up(till Sep 2015)**
ER PR+ve,Her2-ve (Luminal A)	52	70 %	12-On hormone therapy & doing well.
ER PR+ve,Her2+ve (Luminal B)	10	14 %	
ER PR-ve,Her2+ve (Her2 Overexpressing)	4	5%	1-Brain Metastasis

		ER PR-ve,Her2-ve							1-Bone Metastasis	
				8		11 %				
		(Triple Negative)							1-Recurred in opposite breast	

of Luminal B type,7.5% of Her2 overexpression type and 13.4% of Triple negative category.Number of local recurrence ,brain metastasis was highest in Her2 overexpression type whereas lymphnode infiltration and systemic metastasis was more in Triple negative type.The overall survival rate was highest in Luminal A type that is 90.3% and lowest in Her2 overexpressing type &Triple negative type that is 78.8% and 79% respectively.

V. Conclusions

The molecular markers ER,PR &Her-2/neu are amplified in a subset of cancer, are the major driver for tumor cell proliferation and survival. Targeting these pathways therapeutically has remarkably improved the outlook of the patients. The pathologists role in accurately assessing these markers is crucial for successful treatment. The presence of hormone receptor expression in breast carcinoma is associated with good prognostic factors like post menopausal status, small tumor size, negative lymph node status and low histological grading. But the reverse is true for Her2/neu that is associated with adverse prognostic factors like young age, large tumor size, high grade tumors. ER,PR and Her2 are having an inverse association with each other which is explained by estrogen dependent down regulation of Her2/neu pathway. The most common IHC subtype is Luminal A type, shows a better response to hormone therapy because of ER/PR expression. The no. of recurrences and systemic metastasis is more in Her2 overexpressing and triple negative type. Diagnostic assessment of ER/PR and Her2 by IHC should follow the established guidelines and procedure to avoid misclassification of patients who might not otherwise receive life saving treatment.

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