International Journal of General Medicine and Pharmacy (IJGMP)
ISSN 2319-3999
Vol. 2, Issue 1 Feb 2013 1-8

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

STUDY OF ER, PR, HER2neu ON MALIGNANT BREAST LESIONS & CORRELATION WITH OTHER PROGNOSTIC PARAMETERS

SUDHASMITA RAUTA[1] & JAYASHREE RATH[2]

[1]Post Graduate, Department of Pathology, SCB Medical College, Cuttack, Odisha, India

[2]Professor, Department of Pathology, SCB Medical College, Cuttack, Odisha, India

ABSTRACT

Breast cancer is a leading cause of cancer related mortality in Asian countries. Management of breast cancer depends on several tumour and patient related factors. The aim of this study is to determine if any correlation exists between estrogen receptor(ER), progesterone receptot(PR) & Her-2/neu overexpression, grade,tumor size and lymph node status in carcinoma breast.A total 5o 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 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 that 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).

Assessing the relationship between these molecular markers, most hormone receptor positive cases(85%) showed Her2/neu negativity. 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. Assessment of molecular markers in breast cancer is strongly recommended to provide best therapeutic options.

KEYWORDS: Breast Cancer, Estrogen, Her2/neu, Progesterone

INTRODUCTION

Carcinoma breast is the second most common malignant tumor and the leading cause of death in females.[1]There is difference in survival and mortality in patients breast cancer with similar clinicopathological features. This is because of difference in prognostic factors. Clinicopathologic variables like tumor size, histologic grade, nodal metastases, mitosis,necrosis, age may help in predicting the prognosis[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 predictors of response to therapy.

Basing on above three molecular markers breast carcinoma can be divided into four subtypes (IHC Classification)[4](**Robbins,8th ed**)Luminal A

1. Luminal B
2. Her-2/neu Overexpressing
3. 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]**.**

The present study was designed to establish the relationship between the molecular markers and clinicopathologic factors .It also aims to analyse the prevalence of patients in different IHC subtype & their prognosis.

MARERIAL & METHOD

The present study was conducted in the Department of pathology, S.C.B Medical College,Cuttack from September'2009 to September'2011. Histopathological examination of a total of 50 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.

OBSERVATION

Among the 50 cases included in this study Infiltrating duct carcinoma (IDC NOS) was the largest group, accounting for 86% (43/50) of all the cases which is similar to the finding of **Azizun etal**[8] who found the predominant

morphology(85.3%) to be IDC . Besides IDC a ductal carcinoma in situ(DCIS), a Lobular carcinoma in situ (LCIS),a Medullary carcinoma, a Metaplastic carcinoma, a Mucinous carcinoma had been also included. Among the study group,the common age group affected to be 40-59 yrs i.e 78%(n=39). **Kamil etal**[9] found the commonest age group to be affected is 40-49 year whereas **Azizun etal**[8] reported the mean age of the patient was 48.3yrs having breast carcinoma.

Regarding the hormone receptor status present study revealed that the majority of the patients were ER PR+ve ,(31/ 50 i.e.62%),20%(10/50) were ER-vePR+ve,12%(6/50) were ER PR-ve and only 6%(3/50) were ER+vePR-ve. Among the total ER PR positive cases 45%(14/31) belonged to 40-49 years age group, similarly among ER PR negative patients 50%(3/6) were of 40-49 years of age. Comparing the ERPR status with tumor size(**Table 1**) at presentation, it was observed that 77.41%(n=24) of ER PR+ ve cases were at T_2 stage, 16.12% were at stage T_1 and only 6.4% were at T_3 stage.whereas in ER PR –ve group 66.66% were at stage T_2 and 16.66% each in T2 and T3 respectively,while there was none in T_1 stage.

Correlating the nodal status and hormone receptors(**Table 2),** we found 45.16% patients of ER PR+ve group were without any lymph node involvement whereas in ER PR-ve group 99% of the patients had nodal involvement in the form of either N_1 or N_2. 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 are poorely 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%(3/4) , whereas 90% (18/20) of 50-59 yrs and 86%(6/7) of above 60yrs were associated with Her2/neu negativity. Our study observed 75%(15/20) patients with N_0 stage had Her2/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%(3/5) and 100%(1/1) cases showed Her2/neu positivity respectively. Correlating the Her2 status with histological grading(**Table 5**), 100%(n=5) grade I tumors had Her2 negative status but moving on to the higher grade,75%(3/4) of grade III tumors were associated with Her2 positivity.

Correlating the Her2/neu with hormone receptor status(**Table 6**), present study found that 85%(29/34) ER+ve patients showed Her2 negativity,56%(9/16) ER-ve tumors showed Her2 positivity but only 15%(5/34) ER+ve patients had Her2 positive status.Similar result was found with progesterone receptor i.e.89%(8/9) PR-ve tumors showed Her2 positivity whereas only 15%(6/41) 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 Status	Tumor Size								Total No.
	T_1		T_2		T_3		T_4		
	No.	%	No.	%	No.	%	No.	%	No.
ER+ve PR+ve	5	16.1	24	77.4	2	6.4	0	0	31
ER+ve PR-ve	0	0	3	100	0	0	0	0	3
ER-ve PR+ve	1	10	7	70	2	20	0	0	10
ER-ve PR-ve	0	0	4	66.7	1	16.7	1	16.7	6
Total	**6**	**12**	**38**	**76**	**5**	**10**	**1**	**2**	**50**

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

ER/PR Status	LYMPH NODE								Total No.
	N_0		N_1		N_2		N_3		
	No.	%	No.	%	No.	%	No.	%	No.
ER+ve PR+ve	14	45.2	16	51.6	1	3.22	0	0	31
ER+ve PR-ve	0	0	2	66.7	1	33.3	0	0	3
ER-ve PR+ve	6	60	3	30	1	10	0	0	10
ER-ve PR-ve	0	0	5	83.3	1	16.7	0	0	6
Total	**20**	**40**	**26**	**52**	**4**	**8**	**0**	**0**	**50**

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	5	16.1	24	77.4	2	6.4	31
2	ER+ve PR-ve	0	0	2	66.7	1	33.33	3
3	ER-ve PR+ve	0	0	10	100	0	0	10
4	ER-ve PR-ve	0	0	5	83.3	1	16.66	6
Total		**5**	**10**	**41**	**42**	**4**	**8**	**50**

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

Tumor Size	Her2/neu +ve	%	Her2/neu - ve	%	Total
T_1	0	0	6	100	6
T_2	10	26	28	74	38
T3	3	60	2	40	5
T_4	1	100	0	0	1
Total	**14**	**28**	**36**	**72**	**50**

Table 5: Correlation of Her2 Staus with Istological Grading

Histological Grade	Her2/neu +ve	%	Her2/neu - ve	%	Total
G1	0	0	5	100	5
G2	11	27	30	73	41
G3	3	75	1	25	4
Total	**14**	**28**	**36**	**72**	**50**

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

ER Status	Her2/Neu +Ve	%	Her2/Neu -Ve	%	Total
ER –ve	9	56	7	44	16
ER +ve	5	15	29	85	34
Total	14	28	36	72	50
PR -ve	8	89	1	11	9
PR +ve	6	15	35	85	41
Total	**14**	**28**	**36**	**72**	**50**

DISCUSSIONS

The present study was designed to evaluate the various prognostic factors of breast cancer.The prognostic factors which were taken into account were age, the size of the tumour , lymphnode status, histologic grading and expression of hormone receptors and Her2/neu.

Barnes etal 1993[10] in their study showed that age was not related to ER PR status which is consistent with our study that 45% ERPR+ve and 50% ERPR-ve belonged to 40-49 yrs of age. The data obtained in our study was statistically insignificant. **Hawkins etal 1980** [11] were of view that ER+ve tumors were found in 61% of postmenopausal patients. Our study is consistent with the above study as 68.29% of postmenopausal patients were ER PR+ve while only 33.33% of premenopausal patients were having ER PR positivity.

Barnes etal 1993 [10] revealed that ER+ ve tumours were smaller than ER-ve tumour, while **Allegra etal 1978**[12] found no correlation between hormone receptor positivity and size of the tumour.Our study is consistent with **Barnes etal 1993**[10], that 77.41% ER PR+ ve tumors were at T_2 stage, whereas in ER PR –ve group 16.66% each presented with T2 and T3 stage respectively**. Allegra etal 1978** [12] in their study showed that ER positive group patients had a high proportion of node negative patients. While **Fatima etal2009**[13] found no significant correlation between ER PR status and lymph node metastasis. Our study is consistent with **Allegra etal 1978**[12] 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 N_1 or N_2.

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 tumor which is similar with finding of **Azizun etal 2008**[8] who reported 55.3% tumors belonging to grade II.Present study revealed that majority(77.4%) of ER PR +ve are moderately differentiated whereas only 6.4% belong to grade III.In comparison to ER PR+ve tumors 16.6% ER PR-ve cases are poorely differentiated. **Barnes etal 1993**[10] and **Ratnatunga etal2007** [14] in their study on relationship between hormone receptor status and ductal carcinoma concluded that ER positivity decreases significantly for high grade tumour. **Barnes etal 1993**[55] 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 2008**[8] who found Her2 positivity in 24.7% of cases.**Ivkovic etal 2007**[15] reported Her2/neu protein overexpression in 20% of patients. 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 2010**[9] did not find any association between age at

diagnosis and Her2 status.In comparison to above study **Azizun etal 2008**[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 N_0 stage, 69% and 75% of N_1 & N_2 stage showed Her2 negativity respectively..**Kamil etal 2010**[9] and **Ivkovic etal**[15] also did not find any correlation between Her2 and lymphnode infiltration.But **Azizun etal 2008**[8] reported Her2/neu to be positively associated with lymphnode metastasis.Present study found that in case of patients with small tumor size(T_1 stage) ,100% cases belonged to Her2 negative status. On the other side as the tumor size goes on increasing that is the patients with T_3 & T_4 stage, 60% and 100% cases showed Her2/neu positivity respectively. The result is consistent with that of **Ivkovic eta 2007**[15] and **Azizun etal 2008**[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 (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. This finding is comparable with that of **Ivkovic etal 2007**[15] who found all grade I tumors were having Her2 negative status whereas 41% of GradeIII tumors had Her2 positivity.**Kamil etal 2010** [9] in his study did not find any correlation between histological grading and Her2/neu status.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 eta 2007**[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 2008**[8] who found 83.8% ER+ve and 91.9% PR+vetumors with Her2/neu negative status.The above data concluding an inverse correlation between Her2neu and hormone receptors.

In the present study patients are categorized according to the above mentioned molecular markers i.e. the IHC classification(**Table 7**) which includes four category,Luminal A type(ERPR+ve,Her2-ve), Luminal B type(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 2011)
ER PR+ve,Her2-ve	26	70%	12-On hormone therapy & doing well.
(Luminal A)			
ER PR+ve,Her2+ve	5	14%	
(Luminal B)			
ER PR-ve,Her2+ve	2	5%	1-Brain Metastasis
(Her2 Overexpressing)			
ER PR-ve,Her2-ve	4	11%	1-Bone Metastasis
(Triple Negative)			1-Recurred in opposite breast

Adedayo A etal 2009[16] in their study of 1134 breast cancer patients found 68.9% of Luminal A type,10.2% 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.

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 histologcal 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 reccurences 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.

REFERENCES

1. Indian Journal Cancer 2008.Vol 45,Issue 1.
2. Rosai and Ackerman's Surgical Pathology,Rosai,10th edition,2011.
3. Quiet CA , Ferguson DJ et al : Natural history of node negative breast cancer , astudy of 826 patients with longterm followup. J of Clinical Oncol 13 : 1144-1151, 1995.
4. Robbins and Cotran; Pathologic Basis of Disease,8th Edition,p 1084-86.
5. McGuire WL et al: Current status of estogen receptor in human breast cancer.Cancer 36 : 638-44,1975
6. Carolina et al : HER 2 Biology,Detection and Clinical Implications.Arch Pathol Lab Med.2011;135:55-62.
7. R A Walker et al :Immunohistochemical markers as predictive tools of breast cancer.J Clin Pathol 2008;61:689-696.
8. *Azizun-Nisa et al:Comparison of ER,PR and Her2/neu reactivity pattern with histological grade,tumor size and lymphnode status in breast tumor.Asian Pacific Journal Cancer preview,9,553-6,2008.*
9. Kamil M et al:Association between Her2/neu overexpression and clinicopathologic parameters of breast cancer in Northen Malaysia.Ceylon Medical Journal;Vol.55,No.1,March 2010.
10. *Barnes MD et al:Immunohistochemical evaluation of mammary carcinoma-an important new independent indicator of prognosis?Human Pathol 24:469-476,1993.*
11. *Hawkins RAet al :Estrogen receptor and breast cancer ,current status.Br J Surg 67: 153-169,1980.*
12. *Allegra JC et al: An association between steroid hormone receptor and response to chemotherapy in patients with metastatic breast cancer. Cancer 38: 4299-4304,1978.*
13. Fatima et al:Clinical studies on hormonal status in breast cancer and its impact on quality of life.J of Cancer science and Therapy 2:83-89,2009.
14. *Ratnatunga N et al: Hormone receptor expression and Her2/neu amplification in Breast carcinoma in a cohort of Srilanka.Ceylon Medical Journal;Vol.52,No.4,December 2007.*

15. Tatjana Ivković-Kapicl et al : HER-2/neu overexpression in invasive ductal breast cancer – an association with other prognostic and predictive factors**.** Arch Oncol 2007;15(1-2):15-8.
16. Adedayo et al:Breast Cancer SubtypesBased on ER/PR and Her2 expression:Comparison of Clinicopathologic Features and Survival.Clinical Medicine & Research vol 7,No.1/2:4-13,2009