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Correlation of p53 Protein Accumulation and HER2/neu over expression with Clinicopathological Characteristics in Carcinoma Breast in Indian Population

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ABSTRACT

Introduction: Breast cancer is the most common cancer in women and leading cause of cancer death worldwide. Its incidence is rapidly increasing in India also. Present study was aimed to study expression of p53 protein accumulation and Her-2neu over expression in breast carcinoma and its correlation with ER PR status and with various clinico-pathological aspects.

Method: The present study was carried out in the Department of Surgery and Pathology, Institute of Medical Sciences, Varanasi, from the period of July 2011 to June 2013. Routine H&E staining for histological diagnosis and IHC analysis for p53 and Her-2neu was carried out in all cases.

Result: This study includes total 76 cases of breast malignancies including two male patients. Significant correlation was observed between p53 protein accumulation and Her-2/neu expression, with tumor size, grade, stage, pN and hormonal receptors (p value < 0.05). No significant association was seen with histological type, site &side of tumor and menopausal status (p value >0.05).

Conclusion: Our study indicates higher rates of positive expression of p53 and Her-2/neu in breast carcinoma and strongly correlated with various clinic-pathological aspects and hormonal receptor status. Assessment of prognostic markers for the clinical management of breast cancer patients is strongly advocated to provide best therapeutic options. All these adverse features cumulatively suggest about the poor prognosis in our patients. So early detection should be promoted by prompt screening programs and aggressive approach should be adopted along with proper evaluation of all prognostic factors.

Key words- Biomarkers, Immunohistochemistry, Estrogen receptors, Progesterone receptors

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1. INTRODUCTION

Breast cancer is the most common cancer diagnosed in women worldwide and requires multidimensional approach and multimodality treatment (Murray and Lopez, 1997). It is a multifactorial disease, and presumably it represents a complex interplay of genetic susceptibility and environmental factors (McKeown, 1999). Early detection of breast cancer greatly increases the chances for successful treatment options disease free interval and a better prognosis. In India breast cancer is the second leading cause of morbidity and mortality after carcinoma cervix however, in urban areas it is the most prevalent malignancy (25-30% of all malignancy) in women with continuously increasing incidence rate (Harrison et al. 2010).

Estrogen and progesterone are essential hormones for breast growth and their receptors (ER and PR) are established predictive factors for breast cancer treatment efficacy and survival advantage. ERs are transcription factors, which mediate the action of estrogens, whereas the PR gene is regulated by estrogen, and so the PR could be a marker of estrogen action in breast cancer (Payne et al. 2008).

Over-expression of the HER2/neu gene is associated with rapid tumour growth, increased risk of recurrence after surgery, poor response to conventional chemotherapy and shortened survival (Munjal et al. 2009) whereas P53 is associated with cell proliferation regulation, including apoptosis, and in promoting chromosomal stability. It had been demonstrated that some percentage of breast cancers have mutations in tumor suppressor gene p53, which are associated with clinical aggressiveness (Otsuka and Ishioka, 2007).

The identification of triple-negative breast cancer (TNBC), defined as lacking ER, PR, and HER-2 over expression, represent approximately 15% of all breast cancers (Lund et al. 2009), has given rise to a great deal of interest and discussion due to its distinct clinical presentation, innate aggressive biological characteristics and lack of target therapy.

Although prognostic and predictive value of these four biomarkers has been well established in previous investigations however, relation between these biomarkers and clinical characteristics of breast cancer is still obscure and need to be evaluated further. Several available studies have been carried out to analyze the role of ER, PR, Her2/neu and p53 in patients' survival (Naeem et al. 2008) but less number have focused on the relation of these biomarkers with important prognostic factors such as age, tumor size, histological subtype, grade, lymph node status study, which may help in rapid diagnosis and planning treatment. The present study was aimed to elaborate the pattern of expression of hormone receptors, Her2/neu and p53 protein over expression in invasive breast carcinoma in Indian population and to establish its correlation with various clinico-pathological characteristics of breast cancer including age, tumor size, lymph node invasion, tumor type and tumor grade.

2. MATERIALS & METHODS

2.1. Selection of patients

We have carried out a prospective study on 76 histologically proven carcinoma breast patients from the surgical ward of University Hospital, Institute of Medical sciences, Banaras Hindu University. Present study was carried out in the Department of General Surgery in collaboration with the Department of Pathology from July 2011 to June 2013. The clinical and pathologic staging of breast cancer was done according to the TNM-UICC classification (Greene et al. 2006) and all finding were noted down in the prepared proforma. All patients underwent modified radical mastectomy (MRM) after complete work up. Exclusion criteria for enrollment in the study group were patients with clinical or pathologic evidence of cancer at any other site or of having received any type of neo-adjuvant therapy and pregnancy. None of the patients were under oral contraceptives and hormonal therapy. The study protocol was approved by the ethical committee of the Institute of Medical Sciences, Banaras Hindu University. Informed consent of each patient and healthy volunteers was obtained for induction into purely scientific enquiry necessitating tissue samples.

2.2. Analytical method

All examined indices were determined in tissue obtained from the study groups. All patients underwent modified radical mastectomy after complete workup. MRM specimen was thoroughly examined and was transferred to department of Pathology and kept overnight in formalin. Tumor tissue contained in paraffin blocks were sectioned into 3-6 micron section and hematoxylin & eosin staining done for routine histological diagnosis according to Nottingham modification of Bloom Richardson grading system of WHO. Tissue parafiin blocks were used for HER2neu and p53 protein assessment by immunohistochemistry technique. IHC kit of Biogenix company, containing all the reagents along with primary and secondary antibodies was used to study HER2 Neu and p53 expression.

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2.3. Procedure

Sections of 3-6 mm were cut using microtome from paraffin blocks and mounted on glass slide. These sections were baked at 58^0 C for 30 min. Again these sections were deparaffinised in xylene for 5 min. Then those sections were rehydrated in 100% and 95% ethanol for 3 min. It was done thrice and then rinsed with deionized water. Antigen retrieval was done with heat method and after cooling to room temperature again rinsed with deionized water. The sections were placed in 3% H_2O_2 block for 30 min. after which it was washed in water and placed in phosphate buffered saline for 5 min. Primary antibodies were diluted to a concentration of 1:3200 and sections incubated overnight and the secondary antibodies were applied followed by streptavidin conjugated with horse radish peroxidise (HRP). Finally colour was developed by treatment with diaminobenzidine (DAB) solution. After ringing counterstained with hematoxylin for 30 sec. Brown colour was obtained and its intensity was measured. On the basis of intensity of colour produced and percentage of cell stained scoring was done. Test guidelines were used for scoring HER2 Neu and p53 scoring (Table 1, Table 2). Allred scoring system was used for ER/PR receptor estimation (Table 3).

2.4. Statistical analysis

Database management and all statistical analyses were performed using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL). All categorical variable including clinical and histopathological parameters were compared by Chi-square test. Data were analyzed statistically by independent Student's t- Test and $p<0.05$ was considered statistically significant.

3. OBSERVATIONS

40.8 %, 52.6% and 6.6% of the carcinoma breast patients belonged to age between 20-40 years, 40-60 years and >60 years. At the time of diagnosis, 42.1% of the patients were pre- menopausal and 42.1% of females were having tumor at right side. Upper-outer Upper-inner, Lower- outer, Lower-inner, Central 31(40.78%), 13 (17.10%), 11(14.4%), (Table 4).

3.1. Clinico-pathological staging of carcinoma breast patients

In the present study 39.5% patients were in stage II, while 46% were in stage III. We have not found any patient with stage I and metastasis. None of the patient had tumour size less than 2 cm, 53.9% had tumour with diameter between 2 to 5 cm, and 46.1% had tumour with diameter more than 5 cm. 64 patients have clinically mobile node in axilla while in 12 clinically negative axilla was found. 97.4% of the patients were having ductal carcinoma while lobular carcinoma comprised 20 % of the group. Grade 1, 2, 3 breast cancer was found in 23.68%, 57.89% and 18.43% patients respectively. 15 of the patient i. e. 19.7% had no lymph metastasis, while 31 patients i.e. 40.8% had 3 positive axillary lymph nodes. 38.16% of patients had positive while other 61.84% had negative estrogen receptor status. 31 samples i.e. 40.79% showed positive progesterone receptor status (Table 5).

3.2. HER2-neu and p53 protein expression status

51.32% of examined pathological specimens showed overexpression of HER2 neu whereas 48.68% of the sample size has not shown any overexpression for HER2 neu. Similarly immunohistochemical examination of sample size showed that 47.4% of the tumor having overexpression for p53 while 52.6% of breast tumor sample did not show any overexpression for p53 (Table 6).

3.3. Correlation between various parameters for p53 and HER2-neu expression using chi-square ($\chi 2$) test

Correlation study using chi-square ($\chi 2$) test revealed a significant correlation between p53 protein accumulation and tumor size, tumor grade, pathological lymph node, ER, PR and HER2-neu status. However there was no significant correlation between p53 protein accumulation and Menopausal status. This study also revealed a significant correlation between HER2-neu expression and tumor size, tumor grade, pathological lymph node, ER, PR and HER2-neu status. However there was no significant correlation between HER2-neu expression and Menopausal status (Table 7).

4. DISCUSSION

Several histopathological features have prognostic significance in breast carcinoma. This includes histological subtype, grade, lymph node status, estrogen and progesterone receptors status, growth factor and its receptors, proliferation activity and DNA content, oncogenes and tumor suppressor genes (Naeem et al. 2008).

Several biomarkers are being studied intensively as they are valuable in predicting the outcome of the disease and are helpful in deciding the optimal treatment in a particular patient (Glissmeyer, 2007).

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The present study, carried out on 76 pateints of carcinoma breast in the Institute of Medical Sciences, Banaras Hindu University has evaluated p53 protien accumulation and HER2 neu overexpression. Further a correlation has been established with clinicopathological factors as age, menopausal status, tumor size, lymph node status, stage, histological tumor type, tumor grade and ER, PR status.

In our study group, the average age of patients ranged from 20-75 years, mean being 44.28 years, which is a decade lower than the western countries as compared by Stead et al., in 2009 and Sandhu et al., in 2010. Carcinoma male breast were only two in this study group. 42.1% were premenopausal and 57.9% were post-menopausal, which is comparable to a study by Butt et al., in 2012 on 450 patients. Majority of our patients belonged to O^{+ve} blood group (40.78%). B^{+ve} blood group in 21% and A^{+ve} blood group being least common (17%) which is in contradiction to a study by Micheal S *et al* (2009) in which maximum (46.6%) patients belonged to A^{+ve} blood group[14]. 57.9% patients were having a left sided tumor, the main bulk being in the upper outer quadrant of the breast.

All our patients presented with the tumor size of more than 2 cms. Which is in concordance with the studies by Nisa et al., (2008) and Rashes et al., (2007) where >90% patients presented with >2 cm tumor size. The time of presentation is much earlier and size much smaller (<2 cm size) in 71.4% of cases which could be attributed to cancer detection programmes in the western world (Onitilo et al. 2009). We divide our patients into two subgroups of 2-5 cm and tumor more than 5cm. 53.9% of patients belonged to former and 46.1% belonged ti the later group. 60.5% of the patients had stage 3 disease at the time of presentation and 39.5% patients fell into stage 2 disease. 12 patients (15.8%) had clinically no axilla but on histopathological exam 15 patients (19.7%) were p No. 64 patients were clinically N_1 while on HPE, 39.5% had pN_{2a} (>3 axillary nodes), similar to other Asian studies (Sandhu et al, 2010 and Vaidyanathan et al, 2010). This is in little difference from studies in developed countries where majority of patient's presents with a node negative axilla (Huang et al. 2005).

Histopathology in majority (97.4%) showed infiltrating duct Ca and only few (2.6%) had lobular carcinoma, which is comparable to study by Nisa et al., in 2008 and Taucher et al., (2003). In Western population, incidence of lobular cancer is reported to be around 15%[21]. 57.89% of patients had grade 2 tumor and 23.68% had grade 1 and 18.43% had grade 3 tumors which is comparable to studies by Sandhu et al., (2010), Vaidyanatham et al., (2010) and Vasudha et al., (2012).

In Asian population, ER and PR positivity is relatively lesser than in Western population. 38.16% of patients were ER positive and 40.79% were PR positive in our study which is similar to a study by Munjal et al., in 2009 done on 107 patient of Intraductal carcinoma which showed 41.41% positivity for ER and PR. In a Western study by Shapochka et al. (2012), 23 on 62 patients, 76% were positive for ER and 68% for PR (Shet et al. 2009) and found ER positivity in 63% and PR positivity in 57%.

In our study HER 2 neu over expression was seen in 51.32% which is similar to study by Moradi Marjaneh et al., (2008) on 339 patients with HER 2 neu overexpression in 51.7% patients. Western studies by Haung et al. (2005), and Moses Ambroise et al. (2011), showed over expression 17.7% and 27.10% respectively.

p53 protein accumulation was seen in 47.4% of our patients, which is similar to study by Hussain et al. (2011) which showed p53 protein accumulation in 48.9% of their patients. Hence, a relatively low ER, PR positivity and higher HER 2 neu over expression and p53 protein was found in our study.

In our study, HER 2 neu overexpression is associated with lager tumor size (>5cm) in 65.7% of patients which was found to be statistically significant ($p = 0.02$). It was also found to be associated with positive axillary node ($p = 0.025$). Side of tumor, histological type and menopausal status were not found to be associated with HER 2 neu overexpression which is comparable to study done by Rashed et al. (2007) on 50 CA Breast patients.

p53 proteins accumulation was found in 47.4% which was found to be slightly higher than Western population. P53 protein accumulation shows significant correlation with tumor size ($p = 0.003$). Number of positive lymph node ($p = 0.045$) and an inverse association was found between p53 accumulation and ER, PR status.

p53 protein accumulation was seen in 66.67% HER 2 neu overexpressed samples which was statistically significant ($p = 0.011$), similar to study by Fitzgippons et al. (2000), and Rashed et al. (2007), but no such association was seen in study by Hussain et al. (2011).

The percentage of triple negative breast cancer was 14.6% in our study which is comparable to Lund et al. (2009).

The limitation of this study is a short follow up to judge the prognostic significance of these biomarkers relation to disease free interval and overall survival in breast cancer patients. Whether the expression of these receptors (ER and PR) is a direct corollary to the grade and stage of tumor, need to be answered. Does the expression change with downgrading of the tumor with neo adjuvant chemotherapy also need to be evaluated?

5. CONCLUSION

Our experience correlates the p53 protein accumulation and HER 2 neu overexpression with larger tumor size, higher stage, poor differentiation and axillary lymph node involvement.

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SUMMARY OF RESEARCH

1. Carcinoma of the breast is the third most common life-threatening malignancy in women worldwide. However with better investigation tools, better understanding of tumor biology and increasing public awareness more number of cases are being diagnosed at earlier stage still it accounts for the highest morbidity and mortality in females
2. These findings suggest an important role of ER, PR and HER2/neu in the initiation and development of malignant breast disease
3. Present study not only establishes the role of these parameters in malignancy but also provide insight into relationship between the pattern of expression of hormone receptors, Her2/neu and p53 protein with various clinico-pathological characteristics of breast cancer including age, tumor size, lymph node invasion, tumor type and tumor grade.

FUTURE ISSUES

1. Determination of these parameters may be useful not only in evaluating patients with malignant tumors of the breast but also to establish pathogenic stages of breast cancer and should be employed to inform the design and outcome measures of clinical trials.
2. Whether the expression of these receptors (ER and PR) is a direct corollary to the grade and stage of tumor, need to be answered.
3. Does the expression change with downgrading of the tumor with neo adjuvant chemotherapy also need to be evaluated?

CONFLICT OF INTEREST

None of the author has any financial or personal relationship with organizations that could potentially be perceived as influencing the described research. All authors have read the journal's policy on disclosure of potential conflicts of interest.

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Table 1

HercepTest Guidelines for Scoring of HER2neu

Score to Report	HER2 Protein Overexpression Assessment	Staining Pattern
0	Negative	No staining is observed, or membrane staining is observed in <10% of the tumor cells.
1+	Negative	A faint/barely perceptible membrane staining is detected in >10% of tumor cells. The cells exhibit incomplete membrane staining
2+	Weakly positive	A weak to moderate complete membrane staining is observed in >10% of tumor cells.
3+	Strongly positive	A strong complete membrane staining is observed in >10% of tumor cells.

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Table 2

Assessment of p53 expression

Score to report	P53 protein accumulation assessment	Staining pattern
0	Negative	No staining is observed
1	Positive	A nuclear staining is detected in <10% of tumor cells.
2	Positive	A nuclear staining is detected in 10%-50% of tumor cells.
3	Positive	A nuclear staining is detected in >50% of tumor cells.

Table 3

Allred scoring system for ER/PR receptor estimation

Proportion of tumor cells with positive nuclear staining	PS Observation	Average intensity of all positive tumor cells	IS Observation
0	None	0	None
1	> 0 to 1/100	1	Weak
2	> 1/100 to 1/10	2	Intermediate
3	> 1/10 to 1/3	3	Strong
4	> 1/3 to 2/3		
5	> 2/3 to 1		

Total score = PS+ IS

Sum of Proportion Score (PS) and Intensity Score (IS)	Interpretation
0-2	Negative
≥3	Positive

Table 4

Clinical characterstics of carcinoma breast patients and controls

Epidemiological characterstics	Carcinoma Breast (n=76)
Age (years)	
20-40	31 (40.8%)
40-60	40(52.6%)
>60	5(6.6%)
Menopausal status	
Pre menopausal	32 (42.10%)
Post menopausal	44 (57.90%)
Blood Group	
O+ve	31(40.78%)
B+ve	16(21%)
AB+ve	6(7%)
A+ve	13(17.1%)
Other	10(13.1%)

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Tumor side	
Right	32 (42.1%)
Left	44 (57.9%)
Tumor quadrants	
Upper-outer	31(40.78%)
Upper-inner	13 (17.10%)
Lower- outer	11(14.4%)
Lower-inner	4(5.2%)
Central	17(22.36%)

Table 5

Clinico-pathological characterstics of carcinoma breast patients

Variable	No. of patients n (%)
Stage	
I	00(00.0%)
II	30(39.50%)
III	46(60.5%)
IV	00(00.0%)
Tumour size	
<2	00(00.0%)
2-5	41 (53.9%)
>5	35(46.1%)
Lymph node status	
N0	12 (15.8%)
N1	64(84.2%)
Histology	
Ductal	74(97.4%)
Lobular	2(2.6%)
Tumor grade	
1	18(23.68%)
2	44(57.89%)
3	14(18.43%)
Pathological lymph node	
0	15(19.7%)
1-3	30(39.5%)
>3	31(40.80%)
ER	
Positive	29(38.16%)
Negative	47(61.84%)
PR	
Positive	31(40.79%)
Negative	45(59.21%)

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Table 6

HER2-neu and p53 protein expression status

Variable	No. of patients n (%)
HER2-neu	
Over expression	39 (51.32%)
No over expression	37 (48.68%)
p53	
Over expression	36(47.4%)
No over expression	40(52.6%)

Table 7

Correlation between various parameters for p53 and HER2- neu expression using chi-square ($\chi 2$) test

Parameters	p53		$\chi 2$ value	p value	HER2-neu		$\chi 2$ value	p value
	Over expression	No overexpression			Over expression	No overexpression		
Tumor size								
2-5	13(36.11%)	28(45%)	8.759	0.003	16(41.03%)	25(67.56%)	5.383	0.020
>5	23(63.89%)	12(55%)			23(58.97%)	12(32.44%)		
Menopausal status	15(41.7%)	17(42.5%)	0.005	0.941*	17(43.59%)	15(40.54%)	0.072	0.785*
Pre menopausal	21(58.3%)	23(57.5%)			22(56.41%)	22(59.46%)		
Post menopausal								
Tumor stage								
II	7(19.4%)	23(57.5%)	11.485	0.001	15(41.03%)	26(67.56%)	6.012	0.020
III	29(80.6%)	17(42.5%)			24(58.97%)	11(32.44%)		
Tumor grade								
1	4(11.11%)	14(35%)	6.180	0.0455	5(12.82%)	13(35.14%)	6.442	0.039
2	25(69.44%)	19(47.5%)			24(61.54%)	20(54.05%)		
3	7(19.44%)	7(17.5%)			10(25.64%)	4(10.81%)		
Pathological lymph node								
0	4(11.11%)	11(27.5%)	7.902	0.019	3(7.69%)	12(32.43%)	7.358	0.025
1-3	13(36.11%)	17(42.5%)			18(46.15%)	12(32.43%)		
>3	19(52.78%)	12(30.00%)			18(46.15%)	13(35.14%)		
ER								
Positive	06(16.66%)	23(57.50%)	13.3878	0.000	09(23.07%)	20(54.05%)	7.721	0.005
Negative	30(83.33%)	17(42.50%)			30(76.92%)	17(45.94%)		
PR								
Positive	10(22.22%)	21(52.5%)	7.361	0.006	10(25.64%)	21(56.75%)	7.611	0.004
Negative	26(77.77%)	19(47.5%)			29(74.35%)	19(43.24%)		
HER2-neu over expression	24(66.67%)	15(37.5%)	6.451	0.011				
no over expression	12(33.33%)	25(62.5%)						

Not significant

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