CORRELATIONSHIP OF HORMONE RECEPTOR STATUS, p53 MUTATION AND c-erb B-2 OVEREXPRESSION WITH NUCLEAR GRADING IN BREAST CANCERS

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ABSTRACT

Thirty cases of infiltrating duct carcinoma of the breast were studied for the expression of estrogen receptor (ER) and progesterone receptor (PR) status; p 53 protein mutation and c-erb B2 overexpression. The results were correlated with the morphological differentiation, as determined by the Nottingham's modification of the Bloom-Richardson system. Hormone receptor positivity was seen in 46.67% cases, whereas p 53 mutation and c-erb B2 overexpression were seen in 50.00% and 60.00% cases respectively. In grade II tumours receptor positivity, p53 mutation and c-erb B-2 overexpression were 57.15%, 42.85% and 52.38% respectively. The corresponding figures for grade III tumours were 33.33%, 83.33% and 66.67% respectively. As grade I comprised only 3 cases no statistical correlation could be observed. Thus we conclude that receptor positivity declined, whereas p 53 mutation and c-erb B-2 overexpression increased, with increase in tumour grade.

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KEY WORDS :Breast Carcinoma; c-erb B-2; p 53; Receptor status.

Introduction

Prognostication has increased in importance with the acceptance of breast conservation surgeries and demonstration of treatment efficacy for adjuvant chemotherapy in disseminated breast cancer. There has been an intense effort to develop reliable indicators of prognostication, with most of the effort being directed to molecular markers with promising biological relevance [1].

Modified Scarff Bloom Richardson (SBR) system of nuclear grading, is assuming more and more importance to prognosticate breast cancer. The biological markers, which have shown promising results, have been hormone receptor status viz. Estrogen Receptor (ER) and Progesterone Receptor (PR), p 53 protein mutation and c-erb B2 overexpression [1,2]. We wanted to find the usefulness of these markers as prognostic indicators for breast cancer. Comparison of these parameters was carried out with nuclear grading of breast cancer.

Hormone receptors have a role in influencing orderly proliferation, differentiation and secretion in normal breast. In cases of carcinoma breast, hormone receptor status is being suitably utilised to guide therapy and also to predict prognosis. Receptor positive patients are found to respond better to Tamoxifen therapy and have a longer disease free interval [1].

Human p 53 gene is located on the short arm of chromosome 17. It puts a brake on cell cycle, thus pushing the cells into a programmed self-destruction sequence (apoptosis), and thereby preventing unruly amplification of damaged DNA. Expression of p 53 is largely confined to neoplastic breast tissue with a more aggressive phenotype and is correlated with a poorer prognosis [3].

HER 2 / neu or c-erb B-2 are proto-oncogenes encoding for certain transmembrane proteins with tyrosine kinase activity, thereby influencing intracellular growth and differentiation. Approximately 25% of primary breast and ovarian tumours overexpress the protein. It correlates with poor survival, early relapse and confers resistance to chemotherapy. It has been associated with poor prognosis, especially in node negative patients [4].

Material and Methods

Patients : 30 cases of carcinoma which were diagnosed and treated in MDTC, CH (SC), Pune and AFMC, Pune were included in the study. Table 1 summarises the clinical character of these 30 patients.

All patients were females with median age 50 years. The youngest patient was 27 year old and eldest patient was of 75 years. 11 patients were premenopausal and 19 were post-menopausal. Maximum number of cases were in stage II (14-46.70%) and III (12-40.00%). Evaluation of nodal status revealed positivity in 19 (63.33%) patients. Histologically, 23 cases (76.67%) were of Infiltrating Duct Carcinoma, no specific Type, 2 cases (6.67%)

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TABLE 1
Clinical and histological characters (n=30)

Characteristic	Number of patients
Clinical stage :	
I	0 (0.00%)
II	14 (46.67%)
III	12 (40.00%)
IV	3 (10.00%)
Tx	1 (3.33%)
Lymph node status :	
Negative	11
Positive (1-3)	15
> 3	4
Histological typing :	
IDC (NST)	23 (76.67%)
Medullary	2 (6.67%)
IDC with EIC	2 (6.67%)
Colloid	1 (3.33%)
Invasive lobular	1 (3.33%)
Secondary (unknown primary)	1 (3.33%)

Tx - tumour size was not known as primary treatment was done earlier

IDC (NST) - invasive ductal carcinoma - no special type

EIC - extensive intraductal component

each were of medullary carcinoma and IDC with EIC; and 1 case (3.33%) each were found to be of colloid carcinoma, infiltrating lobular carcinoma and secondary from unknown primary.

Nuclear grading : Three observers observed all 30 cases independently and then any difference in opinion was resolved by examining the slides jointly on a multihead NIKON-LABOHOPT microscope. Totalling the scores of tubule formation, nuclear pleomorphism and mitotic figure count based on the modified SBR system [5] gave nuclear grading to each case.

Immuno-histochemistry : Four-micrometer paraffin sections were melted, cleaned in xylene and hydrated in decreasing grades of ethanol. After blocking of endogenous peroxidase activity with a solution of hydrogen peroxide, slides were sequentially treated with different primary antibodies for each parameter viz. mouse monoclonal Estrogen Receptor antibody Ab-11 (Clone 1D5) (NEOMARKERS) -1:50; mouse monoclonal Progesterone Receptor antibody Ab-8 (Clone hPra2+hPra3) (NEOMARKERS)-1:100; mouse monoclonal-c-erb B2/HER-2/neu oncoprotein antibody Ab-8 (Clone2-4001)(neomarkers)-1:200 and mouse monoclonal anti-human p53 protein (clone D0-7) (DAKO)-1:100. This

was followed by biotinylated anti-rabbit immunoglobulin and avidin-biotin peroxidase complex. Diaminobenzidine was used as a chromogen in presence of hydrogen peroxide. Slides were then counterstained with Meyer's hematoxylin. All reactions were carried out at room temperature.

Evaluation of results : In case of ER, PR and p 53, nuclear staining characterised by the distinct brown colour of oxidised DAB was considered positive. For c-erbB-2 however, cell membrane and cytoplasmic staining denoted positivity. A section is classified positive when [6] :-

i. at least 10% of tumour cells stain equivocally with immuno-histochemistry.

ii. includes moderate and strong positives, both detected using the x 10 objective. (Fig 1-4).

Results

Hormone Receptors : Table 2 shows that out of 30 cases, 14 cases (46.67%) showed positivity for ER/PR/both. On analysing receptor positivity with tumour grade, it was observed that 12 out of 21 (57.15%) cases of Grade II were receptor positive, as compared to 2 out of 6 (33.33%) of Grade III cases. Since the number of cases in Grade I were small no definite correlation could be obtained for that category. All the results were analysed statistically and it was observed that difference in receptor positivity between grades II and III were statistically significant ($p < 0.05$).

p53 mutation : p 53 staining showed (Table 2) that out of 30 cases, 15 cases (50.00%) were positive. It was observed that as the nuclear grades advanced the positivity for p 53 also showed an increase in the number of cases. Statistical analysis showed that differences between grades II and III were statistically significant ($p < 0.05$).

c-erb B2: c-erb B-2 expression studies by immunostains showed (Table 2) overexpression in 18 (60.00%) cases. As stated before, grade I cases were not considered, but grades II and III showed a statistically significant ($p < 0.05$) rise in positivity for overexpression of this parameter with the advancement in the grades of tumour.

Discussion

The modified Bloom - Richardson score denotes the morphological differentiation of the tumour. Extensive studies conducted by Elston et al [5] and Stierer et al [7] revealed a predominance of higher grades. Our study also revealed similar results with 70.00% in grade II and 20.00% in grade III. Only

TABLE 2
Morphological grading with IHC parameters (n = 30)

Histological grade	Receptor Status		p ⁵³ Mutation		c-erb b-2 Over-expression	
	+	-	+	-	+	-
I	0	3	1	2	3	0
(3)	(0.00)	(100.00)	(33.33)	(66.67)	(100.00)	(0.00)
II	12	9	9	12	11	10
(21)	(57.15)	(42.85)	(42.85)	(57.15)	(52.38)	(47.62)
III	2	4	5	1	4	2
(6)	(33.33)	(66.67)	(83.33)	(16.67)	(66.67)	(33.33)
Total	14	16	15	15	18	12
(30)	(46.67)	(53.33)	(50.00)	(50.00)	(60.00)	(40.00)

(Figures in parenthesis indicate percentage of cases)

Fig. 1 : ER positive nuclear staining (ER x 100)

Fig. 2 : PR positive nuclear staining (PR x 100)

10.00% were in grade I. Considering an interobserver variability of 30-85% as quoted by Haybittle et al [8], our results are comparable.

Positive hormone receptor status has been found to be more responsive to ablative hormone therapy and to ablative surgeries, and also to have a better prognosis. Biochemical assays were marred by problems of false negatives with small tumours, false positives with non-tumour cells, cumbersome tissue transportation and high cost and time factors. This has paved the path for immunohistochemical evaluation, in formalin-fixed paraffin-embedded tissue sections. Possible fallacies were standardisation of fixatives, fixation times, embedding procedures and antigen loss due to fixation. Other grey areas were in the steps of immunostaining, namely antigen retrieval incubation times, diverse sensitivity of available antigen and stain enhancement techniques. Finally, the system of evaluation remained the most essential part of standardisation of the entire procedure [6].

Failure to use the specific clone may result in a functional ER to remain undetected [9]. Thus we had

Fig. 3 : p53 showing positive staining of nuclei (p53 x 100)

Fig. 4 : c-erb B-2 showing positive staining of cytoplasmic membrane (c-erb B2 x 100)

7 cases of ER-PR+. To avoid such controversies, we have quoted similar results with positivity around 75% [6].

Apparently, the receptor negatives (16 cases ie. 53.33%) in our study seemed to be more as compared to others, but on analysing these 16 cases it was found that 13 were of higher grades, 9 showed p53 mutation, 8 showed c-erb B2 overexpression and 4 were positive both for p53 and c-erb B2. On our limited follow up studies 2 cases developed systematic metastasis. These proved that receptor negatives did have a tendency towards poor prognosis. Owing to a small population, definite correlation could not be ascertained but a trend could be demonstrated, as observed by other workers.

To study expression of p53 protein mutation, we chose specific clone of antibodies for the purpose of prognostication. Our result of positivity of 50% and an increasing trend of positivity with increase in tumour grades matched with that of other workers, where a range of positivity of 27-54% has been

quoted [10].

One of our cases revealed p 53 positivity despite being grade I. This particular case was detected to be c-erb B2 positive as well. Apart from this, 6 cases showed positivity for both p53 and hormone receptor. All 6 were high grade tumours (grade II-5 and grade III-1) and 4 cases had nodal positivity. On consulting literature, similar trend was observed [11]. This indicated poor prognosis, and although Tamoxifen was offered to all the patients, a close vigil had to be kept on them for a possible development of resistance to chemotherapy and metastasis. To overcome such controversies, it is recommended that we must study more than one parameter for a better prognostic correlation.

Finally, c-erb B-2 results were analysed. This is a marker of poor prognosis and correlates with higher grades of tumour and developing resistance to chemotherapy [12]. Overall positivity of 60% was observed with an increase in positivity with advancing grades of tumour. Literature showed similar results, with positivity rate of 10-40% [13].

All the 3 grade I cases showed c-erb B-2 over expression. On further analysis, it was seen that none of them showed receptor positivity, 1 showed p 53 mutation, 2 had nodal involvement, 1 was clinically in stage IV with supraclavicular lymph node metastasis and 1 had a family history of carcinoma breast. Thus, overall all these cases had indicators of poor prognosis and it correlated well with c-erb B-2 status also. This further highlighted the benefits of evaluating multiple markers for a better correlation.

Nodal status has remained one of the better, if not the best correlated parameter for patient prognosis till date. Paik et al [14] has stated the importance of c-erb B-2 over expression in case of node negative patients. This subgroup can develop resistance to chemotherapy and can be managed actively by specific neutralising antibody (Herceptin) [2]. Out of the 11 node negatives in our study, 8 showed c-erb B2 overexpression. 2 patients on followup revealed widespread metastasis within 10 months of detection. This consolidated the observation by Paik et al further, that nodal status is not always a definite indicator and grade of tumour alongwith immunohistochemical parameter should be given equal, if not more importance, in predicting the patient prognosis and guiding the therapeutic regime to be adopted.

In conclusion, we have clearly observed in the study of 30 primary breast cancer cases that nuclear grades of the breast tumours based on modified Bloom-Richardson system correlates very well with

positive staining for hormone receptors, p 53 mutation and c-erb B2 overexpression. With increase in grades of tumours there is a definite fall in receptor positivity and rise in p 53 and c-erb B2 overexpression. How these presumed correlates of aggressive tumour behaviour will actually correlate with overall and disease-free survival will however be known in this study over a period of time as we follow these 30 breast cancer cases over 5 to 10 years or so.

References

1. Page DL, Jensen RA, Simpson JF. Routinely available indicators of prognosis in breast cancer. *Breast Cancer Res Treat* 1998;51:195-208.
2. Gasparini G. Prognostic variables in node-negative and node-positive breast cancer-Introduction. *Breast Cancer Res Treat* 1998;51:193-4.
3. Mazars R, Spinardi L, Ben Cheikh et al. P 53 mutations occur in aggressive breast cancer. *Cancer-Res.* 1992;52:3918-23.
4. Allred DC, Clark GM, Tandon AK et al. HER-2/neu in node negative breast cancer : prognostic significance of overexpression is influenced by presence of in-situ carcinoma. *J Clin Oncol* 1992;10:599-602.
5. Elston GK, Ellis IO. Pathological prognostic factors in breast cancer. The value of histologic grades in breast cancer. Experience from a large study with long term follow up. *Histopathology* 1991;19:403-10.
6. Barnes DM, Mills RR : Oestrogen receptors : the history, relevance and methods of evaluation. In : Kirkham N, Lemoine NR, editors. *Progress in Pathology*. Edinburgh : Churchill Livingstone, 1995;89-114.
7. Stierer M, Rosen H, Weber R et al. Immunohistochemical and biochemical measurement of oestrogen and progesterone receptors in primary breast cancer. Correlation of histopathology and prognostic factors. *Ann Surgery* 1993;218:13-21.
8. Haybittle JL, Blamey RW, Elston CW et al. A prognostic index in primary breast cancer. *Br J Cancer* 1987;45:361-6
9. Desai AJ, Luqmani YA, Walters JE et al. Presence of exon 5 deleted ER in human breast carcinoma : Functional analysis and clinical significance. *Br J Cancer* 1997;75(8): 1173-84.
10. Hearslev T, Jacobsen GK. An immunohistochemical study of p53 with correlations to histopathological parameters, c-erb B2, proliferation cell nuclear antigen and prognosis. *Human Pathol* 1995;26:295-301.
11. Walker RA, Dearing SJ, Lane DP et al. Expression of p 53 protein in infiltrating and in situ carcinoma. *J Pathol* 1991; 165 : 203-11.
12. Tandon AK, Clark GM, Chamness GC et al. HER-2/neu oncogene protein and prognosis in breast cancer. *J Clin Oncol* 1989;7:1120-8.
13. Slamon DJ, Clark GM, Wong SS et al. Human breast cancer : Correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177-81.
14. Paik S, Hazan R, Fisher ER. Pathological findings from the NSABP (protocol B-06). Prognostic significance of c-erb B2 protein overexpression in primary breast cancer. *J Clin Oncol* 1990;8:103-12.