Immunohistochemical co-expression of c-erbB-2/Neu oncoprotein, altered tumour suppressor (p53) protein, EGF-R and EMA in histological subtypes of infiltrating duct carcinoma of the breast

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Carcinoma of the breast has an unpredictable biological behaviour. Several oncogenes have been implicated in the progression of breast cancer. Immunohistochemical staining of c-erbB-2 (Neu) oncoprotein and mutant p53 protein on 45 cases of infiltrating duct carcinoma (IDC) of the breast revealed 33% membrane positivity of c-erbB-2 oncoprotein, 46% nuclear positivity of mutated p53 protein, 33% and 84% membrane positivity of EGF-R and EMA respectively. Staining profile of c-erbB-2 oncoprotein in various histological subtypes of IDC of the breast indicated a high positivity rate in comedo followed by NOS and cribriform subtype. Similarly, high incidence of immunopositivity of mutant p53 protein was observed in comedo and cribriform subtypes while papillary carcinoma were found exclusively positive for mutated p53 protein. Interestingly, tubular subtype of IDC was not positive for c-erbB-2 oncoprotein as well as p53 mutant protein. Further, comedo and cribriform subtypes of IDC revealed high grade histological features of tumour of the breast with high mitotic count, presence of marked pleomorphism and multinucleation thus, reflecting a positive relationship with overexpression of c-erbB-2 (Neu) oncoprotein as well as mutant p53 protein. The results on immunoeexpression of c-erbB-2 oncoprotein and mutated p53 protein in various histological subtypes of IDC of the breast demonstrated c-erbB-2 status as an important predictor and also indicated that oncogene product may be involved in growth factor response pathway.

Breast Cancer is the most frequent cancer in women worldwide with more than half a million new cases being reported each year. Carcinoma of the breast is the second most common cancer among Indian women and an increasing trend in its incidence has been observed as per the reports of National Cancer Registry Programme 1988-89.

Numerous epidemiological studies3,4 carried out in breast cancer have identified several risk factors. Breast cancer is rare in males. The incidence curve for breast cancer rises with age from 30 to 70 years, but shows a notable inflexion at around 45-54 years, the age of the menopause, after which the risk increases much more slowly with age. Rates in developed countries are significantly higher than those in less developed countries. Similarly, women in higher socio-economic status are affected more than women of lower socio-economic status. Obese women are at higher risk for breast cancer than women who are not obese. Increased total calories and fat consumption seems to increase the risk6.

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Breast cancer risk is increased by increasing duration of exposure to endogenous ovarian hormones, so early menarche or late menopause increases the risk. Other determinants of risk such as age at first pregnancy and parity likewise implicated endocrine mechanisms but are less convincing. Nulliparity increases risk and high parity decreases risk, at least after age 50. Estrogen replacement therapy and combined oral contraceptive use may cause a small increase in the risk for breast cancer.

A family history of breast cancer increases the risk. This may be attributable to genetic and environmental, similarities among family members. Overall, only 10-15% of breast cancer is ascribable to family history, and about half of this is related to dominantly inherited susceptibility genes. BRCA1, a susceptibility gene for breast cancer, has many characteristics of a tumour suppressor gene. Women who carry BRCA1 have a 85% chance of developing breast cancer. The identification of BRCA1 has focused attention on other genetic loci, some new and others already suspect, including BRCA2, p53, H-ras polymorphisms etc. Among the estrogen-regulated genes, some are also regulated by growth factors viz.
cathepsin D gene. From different studies on cell lines, new immunological and genetic probes have been raised that can be applied to breast cancer tissue to identify the expression of different genes involved in the control of mammary tumours growth and invasion. From different studies on cell lines, new immunological and genetic probes have been raised that can be applied to breast cancer tissue to identify the expression of different genes involved in the control of mammary tumours growth and invasion.  

Various histopathological risk factors identified in breast cancer include histological type of tumour, tumour grade, peritumoral lymphatic and vascular invasion and axillary lymph node metastasis. The rare but favourable histological types of breast cancer include tubular, papillary, colloid and adenoid cystic carcinoma. All other histological types are classified under moderately to highly metastasizing group. Histological grade of tumour has a predictive value in breast cancer prognosis.

In the recent past, c-erbB-2 (Neu) a growth factor related oncogene has been frequently cited as a marker of poor prognosis in breast cancer. The epidermal growth factor receptor (EGF-R) and c-erbB-2, both encode transmembrane proteins with two repeats of extracellular cysteine rich domain and an intracellular tyrosine kinase domain. Recent studies suggest that measurement of the presence of EGF-R, especially in conjunction with c-erbB-2 may be valuable in predicting response to endocrine therapy. Overexpression of c-erbB-2/Neu leads to receptor autophosphorylation and the subsequent activation of kinases substrates involved in the cellular signal transduction mechanisms, which eventually affects the nuclear transcription of genes regulating cell cycle progression.  

Tumour suppressor gene (p53) plays a central role in the negative growth regulation of a wide variety of cells. Mutations in p53 gene results in loss of this negative growth regulation and promotion of malignant cell; p53 alterations are common in breast cancer and are associated with a worse prognosis which is an indirect manifestation of a more aggressive growth.

In this study, attempt has been made to analyse morphological characteristics of infiltrating duct carcinoma (IDC) of the breast alongwith immunohistochemical staining to assess the expressions of c-erbB-2 (Neu) oncoprotein, altered p53 protein and EGF-R.

Materials and Methods

The study was carried out on randomly selected histopathologically proven paraffin embedded archival tissue sections from 45 cases of the infiltrating duct carcinoma (IDC) of the breast alongwith 4 cases of benign breast disease (fibroadenoma) as negative controls obtained from the Department of Pathology, Maulana Azad Medical College, New Delhi.

Paraffin embedded tissue sections (5 μm) were subjected to routine haematoxyline and eosin staining for histopathological diagnosis as well as immunohistochemical staining of c-erbB-2 (HER-2/Neu), EGF-R, p53 mutated protein and epithelial membrane antigen (EMA). The histological grade of malignancy was evaluated on H&E stained sections using.

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Fig 1 — Immunohistochemical expression of c-erbB-2/ Neu oncoprotein in infiltrating duct carcinoma of breast, X 400.
Fig 2 — Immunohistochemical expression of p53 altered protein in infiltrating duct carcinoma of breast, X 400.
modified Bloom and Richardson (BR) grading for breast cancer, assessing each of several histological parameters, viz. subtype, cell size, nuclear pleomorphism, mitotic activity, periductal sclerosis as well as lymphocytic infiltration.

Immunohistochemical staining was performed as described by Ratnakar et al. The murine anti-human monoclonal antibody (McAb) Ab1, directed against the extracellular domain of EGF receptor, murine anti-human (McAb) c-erbB-2 (Neu) clone 3B5 raised against the C-terminal domain of p185 c-erbB-2 (Oncogene Sciences, Manhasset, NY), p53 protein pan (Clone PAB 122, Cat.No.1413147) and Epithelial membrane antigen (EMA) (Boehringer Mannheim Biochemical) were diluted 1:20 in phosphate buffer saline (PBS) and used as primary antibodies to EGF-R, c-erbB-2, p53 protein as well as EMA respectively for Immunohistochemistry. Rabbit anti-mouse Immunoglobulin G (Dakopatts,Copenhagen, Denmark) was used as the secondary antibody (diluted 1:40 in PBS). For detection of immunoreactivity, mouse PAP complex (Dakopatts) as well as 3,3 diaminobenzidine tetra hydrochloride (DAB) (1mg/1µl) substrate with 0.001% H2O2 was used. Intensity of immunoreactivity was scored semi-quantitatively by classifying the sections as no detectable expression (-) or low (+), moderate (+++) or high staining intensity (+++).

Statistical analysis — The distribution of various oncoproteins between different histotypes of breast carcinoma was analysed by utilizing Fisher’s exact test since the number of cases under the group histological subtypes were less than five or zero.

Results and Discussions
The tissue sections exhibiting distinct membrane staining of c-erbB-2 (Neu), EGF-R and EMA or nuclear immunoreactivity for mutated p53 in tumour cells were identified as a positive case (Figs 1 & 2).

Immunohistochemical staining on 45 cases of infiltrating duct carcinoma (IDC) of the breast revealed 33% (15/45) membrane positivity of c-erbB-2 (Neu) oncoprotein, 46% (21/45) nuclear positivity of mutated p53 protein, 33% (15/45) membrane positivity of EGF-R as well as 84% (38/45) membrane positivity of EMA (Table 1). However, none of the four cases of benign breast diseases have shown immunoreexpression of c-erbB-2, p53, EGF-R and EMA. It is interesting to observe that all five cases of comedo subtype of IDC have revealed immunoreexpression of c-erbB-2(Neu) oncoprotein and mutated p53 protein while four out of five cases were positive for the immunoreexpression of EGF-R. On the other hand, NOS subtype of IDC cases have shown an immunopositivity in 27% cases (9/33) for c-erbB-2 as well as for EGF-R, 36% (12/33) positivity for mutant p53 protein. These differences were found to be statistically significant (P<0.05). Further, one out of two cases of cibiform was positive for c-erbB-2 and EGF-R while these both cases were positive for p53 mutant protein. Surprisingly, all cases of tubular and papillary subtype of IDC were found to be negative for expression of c-erbB-2 oncoprotein and EGF-R, while cases of papillary type exclusively revealed expression of mutant p53 protein. 80-100% cases of all subtypes of IDC also shown immunoreexpression of EMA.

Further, comedo and cibiform subtypes of carcinoma of the breast revealed 'high grade' of histological features of tumour with high mitotic count, presence of marked pleomorphism and multinucleation, thus, reflecting a positive relationship with overexpression of c-erbB-2 (Neu) oncoprotein as well as mutant p53 protein, thereby, suggesting c-erbB-2 status as an important predictor
of poor prognosis. It is striking finding of the study to observe that expression of c-erbB-2 oncoprotein was totally absent in tubular and micropapillary subtypes of the IDC having 'low grade' histological features of the breast tumour.

Overall, tumours with high histological grade (n=7) showed 86% (6/7) immunopositivity for c-erbB-2 and 71% (5/7) positivity for EGF-R while all cases were positive for altered p53 immunoposexpression. On the contrary, tumours of low histological grade (n=38) revealed 24% immunopositivity for c-erbB-2 and EGF-R (9/38). 14 out of 38 cases of low grade tumours (37%) were positive for altered p53 protein. These differences were found to be statistically significant (P<0.05). The study was an attempt towards the immunohistochemical analysis on immunoposexpression of c-erbB-2 oncoprotein, mutant p53 protein, putative growth factor receptor (EGF-R) as biological parameter that possibly have significance in morphological differentiation of tumour or to identify high grade features of infiltrating duct carcinoma of the breast.

Different studies have shown that one third to one half of breast tumour tissues are positive for EGF-R by immunohistochemical studies using monoclonal antibodies. In our study, the expression of EGF-R was found to be 33.3% (15/45).

Overexpression of c-erbB-2 oncoprotein is a frequent event in primary breast cancer. Lipponen et al. found that 31% of the tumours were positive for c-erbB-2, whereas, Marks et al. in their study on early stage breast cancer noticed that 17% were positive for c-erbB-2. Overall, the c-erbB-2 gene has been reported to be amplified in 10-30% of most types of human adenocarcinomas including breast cancer. In our study, the expression of c-erbB-2 was 33.3% (15/45).

p53 gene mutations and nuclear accumulation of the p53 protein are events that occur with close relationship in the progression of a breast cancer, and that p53 abnormalities appear to correlate with a high grade of the malignancy. Elledge et al. observed that tumours with altered p53 protein are inherently more aggressive. Chu et al. detected 30.5% immunohistochemical positivity for p53 protein. They also observed that there was significant correlation between p53 protein expression and high histological grade and high mitotic index. van der Keoy et al. demonstrated 27% p53 overexpression in breast cancer. 24% immunopositivity was detected by Chen et al. Whereas Younes et al. observed that p53 positive nuclear staining was present in 30% malignant lesions. However, this study has revealed immunopositivity of p53 altered protein in 46.6% (21/45) cases of breast cancer.

Rosen et al. noticed that p53 was expressed in 23% cases of duct carcinomas. More often, p53 was present in carcinomas with high-grade in their study. Further, they commented that cases with both p53 and c-erbB-2 positivity had bad prognosis. Association between histological grade, mutated p53 protein expression and c-erbB-2 immunopositivity was also observed by many investigators. On the other hand, Charpin et al. expressed their view that p53 expression was independent of c-erbB-2 expression. However, in their study, p53 correlated with high histological grade and it was more often observed in the comedo carcinomas. Similarly, Eriksson et al. found that intraductal carcinomas of comedo type and poorly differentiated invasive carcinomas of comedo type expressed the mutant p53 protein.

Widespread immunoposexpression of c-erbB-2 oncoprotein as well as EGF-R gene products on the infiltrating duct carcinoma (IDC) cases of the breast of the present study can be stated to be correlated with what would be called 'high grade' features i.e. large pleomorphic cells with a high mitotic rate in comedo and cribriform type of IDC of the breast. This overexpression appears to be associated with mutations in p53 gene resulting in a non-functional protein that is accumulated in tumour cell nuclei. However, the immunoposexpression of c-erbB-2 oncoprotein was found to be totally absent in tubular and micropapillary subtype having 'low grade' of histological grade of the IDC of breast. These observations suggest that these variants may have biological as well as morphological differences which might be of prognostic value of these marker for a subgroup of tumours. This could be as a result of some oncogenes only being effective at certain time point in the differentiation of mammary gland.

The present study reflects that the positive relationship of immunoposexpression of c-erbB-2 oncoprotein and p53 protein could be interpreted as having lost one control mechanism of cell proliferation and have gained one activator with malignant potential. Interestingly, all c-erbB-2 positive IDC cases revealed positivity for EGF-R indicating oncogene product may be involved in growth factor receptor response pathway.
Therefore, it can be speculated that concomitant overexpression of c-erbB-2 and p53 could form the basis to identify breast tumours with different biological behaviour.

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References