Hormone Receptors, Her-2/Neu and Chromosomal Aberrations in Breast Cancer

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Abstract

Background: There is a great deal of disparity in the incidence of breast cancer in rural and urban India on one hand and between India and Western population on the other.

Methods: We analysed steroid receptor status in cases of breast cancer in a small sample of patients in armed forces. Infiltrating duct carcinomas of breast recorded histologically in mastectomy specimens in last two years were accessioned in the present study with reference to patient and tumour characteristics.

Result: In contrast to the higher rates reported in western literature, only 33 % of the tumours expressed estrogen receptors (ER) and progesterone receptors (PR), of which 24% were ER positive and 30% PR positive. Negative steroid receptor status did not correlate with presence or absence of metastatic nodes, however it was predominant amongst the high grade infiltrating duct carcinomas in this study. Necrosis and lymphovascular invasion demonstrated an inverse relationship with the ER/ PR reactivity. 70% of the node positive cases expressed Her -2/ Neu, reflecting a higher immunoreactivity in this subset of patients. Aneusomy for chromosomes 1, 11 and 17 was common in node positive cases.

Conclusion: Evaluation of chromosomal aberrations by Fluorescent In Situ Hybridization (FISH) technique correlates well with traditional histological parameters.

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Key Words : Breast carcinoma; Hormone receptors; Her -2/ Neu

Introduction

The approach to managing breast carcinoma has undergone enormous changes over the last 20 years. The choice of conservative and reconstructive surgery today is more popular than mastectomy. Such changes are accompanied by increasing range of systemic, hormonal and cytotoxic drugs used in both adjuvant and neoadjuvant settings. Hormone receptor test is done routinely in these cases since hormone treatment has fewer side effects and it prevents recurrence in 25% of cases [1]. Estrogen receptors (ER) and progesterone receptors (PR) have been accepted as established procedures in the routine management of patients with primary breast cancers along with Her-2/neu (human epidermal growth factor receptor type 2), a genetic alteration second to p53 mutation [2]. Her-2/neu shows over expression in high grade cancers and displays lower responsiveness to hormone receptor modulators. It is an indicator of reduced disease free survival in lymph node positive breast cancers. Accompanying the histologic progression are numerous molecular alterations that result in oncogene activation and/or tumour suppressor, inactivation. Notably gains of chromosomes 1q, 8q, 11q, 13 and loss of 8p 17p 22q

have been reported in breast cancers and loss of few have been associated with poor prognosis in these cases [3].

We studied the distribution of hormone receptor status and its correlation with traditional prognostic parameters as well as Her-2/neu expression in 75 cases of infiltrating duct carcinoma and analysed numerical chromosomal aberrations for chromosomes 1, 11 and 17 in 30 of these cases.

Material and Methods

Seventy five female patients histologically confirmed as infiltrating duct carcinoma of the breast were accessioned in this study. Representative section from the tumour tissue was sliced and fixed in 10 % buffered formalin for 16-18 hours, followed by routine processing and embedding. Paraffin sections of 5 µm thickness were subjected to hematoxylin and eosin stain. Histological assessment of tumour grade (modified Richardson Bloom Scoring system), presence of ductal carcinoma in situ (DCIS), nodal status, desmoplasia, elastosis, calcification, necrosis, and lymphovascular invasion were recorded in each case.

ER / PR and HER-2/neu analysis was carried out as a routine procedure on the paraffin embedded breast tumour sections by immunohistochemical (IHC) method using monoclonal

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antibodies for ER (ER-ID-5D); PR (PgR636); and HER-2/neu (polyclonal anti rabbit cerb -B2 antibody). Positive ER/PR staining was identified in form of dark nuclear staining. Scoring for ER/PR was based on percentage of nuclei stained, as well as intensity of nuclear staining [4]. For Her-2/neu, membrane staining in tumour cells was scored from 1- 3 [5]. Positive and negative controls were included in each batch (Fig 1B).

Chromosome analysis on tumour sections was done using Fluorescent In Situ Hybridization (FISH) technique on poly-L-lysine coated slides using the Path Vysion FISH probe – to detect chromosome 11 (11 alpha satellite DNA probe labeled with spectrum green VYS-32-132011) and chromosome 17 (17 alpha satellite DNA probe labeled with spectrum orange VYS-32-130017). Single color FISH technique was also used to detect chromosome 1 (1 alpha satellite DNA probe labeled with spectrum orange VYS-32-1300011).

Evaluation of numerical chromosomal aberrations by FISH technique using cytological suspensions prepared from archival tissue was done as per recommendations by Halder et al [6]. 30µm thick formalin fixed paraffin embedded sections were deparaffinized in xylene, followed by dehydration in 100% alcohol and subsequently down to water. Subsequently cytological suspensions were made from these sections as per recommended [7]. Smears were prepared by dropping cell suspension from a height on a small area on the clean, chilled glass slides. These were kept overnight at room temperature for ageing, followed by heat denaturation at $73 \pm 2^\circ \text{C}$ and immersion in 70% formamide in 20X SSC (pH 7.0 ± 2) for five minutes. Probes were prepared in dark room as per manufacturers instructions and warmed in water bath at $73 \pm 2^\circ \text{C}$ for five minutes. Probe was applied on to the slide after sealing the edges by rubber cement and hybridization was done at 37°C in hybrite for 16 hours. Post hybridization washing was performed at 75°C x two minute using 2 x SSC with a 0.3% solution of NP 40 followed by 0.1% NP 40X 20 SSC at room temperature for one minute. The slides were dried, counter stained with 4,6 diamino- 2 diphenylindole dihydrochloride (DAPI) and observed under oil immersion using a fluorescent microscope after applying a cover slip and evaluated for specific chromosome by counting 100 tumour nuclei (Fig 1D). Signals from overlapping nuclei were not counted. The benign ductal epithelial cells obtained from normal breast parenchyma served as positive controls.

Results

Majority 44/75 (59%) of cases were postmenopausal women and the ER/PR status showed a higher incidence of reactivity with increasing age (Table 1). Receptor negativity was found to prevail in the menopausal age group. There were 13/20 and 5/9 cases in the age groups of 51-60 years and 61-70 years respectively displaying negative hormone receptor status. Nonetheless, in the age group of 71-80 years, three out of four patients revealed positivity for ER/PR. T2 sized tumours were 73% of the total and the receptor status was noted to be comparatively increased in larger sized tumours than in T1 and T2 group as depicted in Table 2. In this study only 25/75 cases expressed ER, PR or both (three cases only ER+, 12 only PR+, and 10 cases both ER + & PR+) remaining

Fig. 1 : A : H&E section from a grade II infiltrating duct carcinoma (x 400). B : IHC showing ER2+ tumour cells in breast cancer. C : ER positive cells in benign fibrocystic disease of breast (internal control). D : Fluorescent signals for chromosome 1 in cancer cells (FISH technique), with some showing three signals

Table 1
Correlation of age with ER/PR status

Age group (Number of cases)	Positive (%)	Negative (%)
21-30 (4)	1 (25)	3 (75)
31-40 (21)	6 (28.4)	15 (71.4)
41-50 (17)	4 (23.5)	13 (76.4)
51-60 (20)	7 (35)	13 (65)
61-70 (9)	4 (44.4)	5 (55.5)
71-80 (4)	3 (75)	1 (25)
Total (75)	25 (33.3)	50 (66.6)

Table 2
Relationship of ER/PR status with tumour size

T size	Positive (%)	Negative (%)	Total
< 2 cm	2 (28.4)	5 (71.4)	7
2-5 cm	18 (32.7)	37 (67.2)	55
> 5cm	5 (38.4)	8 (61.4)	13
Total	25	50	75

50 cases were both ER and PR negative. Patients were thus divided into the following four groups; ER+PR+ (10/75 or 13 %); ER-/ PR+ (22/75 or 27.33%); ER+/ PR- (13/75 or 18.33%) and ER- PR- (50/75 or 66.66%) as shown in Fig 2. Out of 75 carcinoma cases 57 were grade II type of infiltrating duct carcinoma (Table 3). The reactivity for steroid receptors was observed to decrease with increasing grades. The tumour receptor positivity was more often associated with nodal metastases than in node negative cases. Of the 40 cases with nodal metastases 15 revealed positivity for ER/PR in comparison to only 10/ 35 node negative cases (Table 3). An attempt to evaluate the Her-2/ neu status demonstrated over expression in 43 cases of infiltrating duct carcinoma (Table 4). It increased proportionately with increasing histological grades of carcinoma. 25% of grade I, 59% grade II and 75%

Fig. 2 : Pie diagram depicting distribution of hormone status

grade III carcinomas displayed over expression of Her-2 neu (Table 4). A strong correlation was observed between Her-2 Neu status and nodal metastases, 70% of the node positive as compared to 34.2 % node negative cases expressed Her-2 Neu in this study. Thirty hormone receptor negative cases were taken up for evaluation of numerical chromosomal aberrations by FISH technique (Table 5). Aneusomy was observed in 18, 12, and 14 cases each for chromosomes 1, 11 and 17 respectively. There were six cases in which aneusomy was present in all the three chromosomes. Numerical chromosomal aberrations were common in node positive as compared to node negative cases (Table 6).

Discussion

An immunohistochemical analysis of steroid receptor status and Her-2 neu evaluation was performed in 75 cases of infiltrating duct carcinomas of breast encountered in families of our soldiers. Our results show that the proportion of both ER and PR positive tumours increases with age. The proportional increase in ER and PR positivity with age was more marked for PR than for ER. Low-grade duct carcinoma and those with elastosis expressed steroid receptors more often. The presence of necrosis and lymphovascular invasion showed an inverse relationship with ER PR non-reactivity. High-grade infiltrating duct carcinomas, were predominantly ER PR negative in our study. However, in our study desmoplasia and calcification did not correlate with steroid receptor reactivity.

In published literature [4], approximately 50% of tumours are ER+ PR+, 25% ER- PR-, 20% ER+ PR- and 5% ER-PR+. In contrast, our study reports a high proportion (66.6 %) of receptor negative cases. Desai et al [8], also obtained a high incidence of steroid receptor non-reactivity in breast cancer patients in India. The overall positivity rate for ER of 24% and PR of 30% is lower as compared to other reports, possibly because of the difference in techniques of evaluation [4], high tumour grades and majority being menopausal women in this study. An important limitation of our study was the lack of information regarding other factors that may be associated with hormone receptor status e.g

Table 3

Association of ER/PR status with histological grade and nodal status

ER/PR	I (10.6%)	II (76%)	III (13.3%)	Node Pos	Node Neg
Positive	4 (50)	18 (31.7)	3 (30)	15 (37.5)	10 (28.5)
Negative	4 (50)	39 (68.4)	7 (70)	25 (62.5)	25 (62.5)
Total	8	57	10	40	35

Table 4

Correlation of Her -2/Neu expression with tumor grade and nodal status

Her-2/ Neu status	Grade I (8)	Grade II (57)	Grade III (10)	Node Positive (40)	Node Negative (35)
Positive (43)	2	34	7	31	12
Negative (32)	6	23	3	9	23

Table 5

Numerical chromosomal aberrations

Chromosome 1	Chromosome 11	Chromosome 17	Total
Disomy	Disomy	Disomy	4
Disomy	Disomy	Aneusomy	4
Disomy	Aneusomy	Disomy	3
Aneusomy	Disomy	Disomy	7
Disomy	Aneusomy	Aneusomy	1
Aneusomy	Disomy	Aneusomy	3
Aneusomy	Aneusomy	Disomy	2
Aneusomy	Aneusomy	Aneusomy	6

Table 6

Association of chromosomal aberrations with nodal status

Chromosomes	Node positive	Node negative
Chromosome 1 (18)	14	4
Chromosome 11 (12)	10	2
Chromosome 17 (14)	11	3

data on hormonal and reproductive factors, medical history and family history of breast cancer. Nulliparity/late age at first birth, early age at menarche, higher body mass index and use of hormone replacement therapy have all been associated with increased risk of developing an ER + tumour but with a decreased risk of developing an ER- tumour. Young patients have high levels of circulating oestrogens and a correspondingly low expression of steroid receptors, which is reflected in their tumours. There appears to be a variation in steroid receptor positivity in the Asian population. Known differences in ER and PR status by race, particularly between black and white women are known [9]. Chariyalertsak et al [10] reported similar observations with lower rates of 36.1% ER and 45.8% PR reactivity in breast cancer cases in Thailand. In contrast, one study recorded 73.5% and 65.5% of ER and PR reactivity in their analysis of 3000 breast cancers [11]. It is also intriguing to find a higher proportion of ER- and PR+

(21%) tumours when compared to those documented in literature. The existence of this subgroup can be explained on the basis of artefactual laboratory reporting or possibly genetic alteration, which switches on PR expression resulting in the occurrence of a 'true ER-PR+' phenotype [9]. It has been documented that ER-PR+ patients have a higher frequency of soft tissue and central nervous system metastasis, a lower frequency of bony metastasis and partial responsiveness to tamoxifen treatment as compared to 80% response rate for double positive tumours [2]. Moreover, the S phase fraction in the ER-PR+ subgroup is considered to be significantly higher than that noted in ER+ PR+ and ER+ PR- subgroups [10].

On correlation of ER/PR status with nodal metastases it has been observed that metastatic tumours revealed non-reactivity for hormone receptors more often than primary tumours. Values fluctuate from 30.8-58% in node negative and 20.3 - 33.5% with nodal involvement in breast cancer cases for receptor positivity in various studies [4,8]. In our study, the hormone receptor positivity was apparent in 28.5 % node negative and 37.5% node positive cases, although increased receptor positivity is expected with lack of nodal involvement.

Breast carcinomas cases have recorded 15-20% positivity for Her-2/neu expression in some [5], but the results varied in other studies [12,13]. In the present study, 57.2 % cases exhibited Her-2/neu overexpression which is higher than that reported by other series in India [12,14]. In our study 70% of cases were node positive breast cancers. Scawn et al [9], noted Her-2/neu expression in 28% mostly node negative cases. Others have described nearly 23% cases with Her-2/neu overexpression without any significant difference between the node positive and negative cases [13]. Her-2 neu has been negatively correlated with receptor status [13]. Her-2 neu has significant prognostic value as a marker since it can predict resistance to hormonal therapy. Recently study by Burstein et al [15], attempted to determine whether the addition of trastuzumab to chemotherapy in the neoadjuvant setting could increase pathologic complete response (pCR) rate in patients with Her2/neu positive disease. They correlated its expression not only with relative tamoxifen resistance, but demonstrated sensitivity to aromatase inhibitors, to anthracyclines and possibly to taxanes and dose dense regimens.

Numerical chromosomal aberrations for chromosome 1,11 and 17 in interphase nuclei from 30 paraffin embedded sections were examined. All these cases were negative for both steroid receptors. Twenty six of the thirty carcinomas examined demonstrated aneusomy for any of the three chromosomes (Table 5). Many

studies have demonstrated imbalances for chromosomes 11 and 17 [3], as compared to chromosome 1 [16] in breast cancers. In the present study, aneusomy for chromosomes 1,11 and 17 was common in cases with nodal metastases. No significant relationship has been observed between aneusomy and nodal status in breast cancers in other studies [16]. These observations could be due to variations in the techniques and that studies of this nature on paraffin embedded tissues are few. Paraffin sections have been used by some workers [6], but majority used the technique on cytological material [16,17].

In conclusion, this study reports a significantly high incidence of ER- PR- and ER- PR+ phenotypes in the selected population of breast cancer patients. These observations also suggest that breast cancers seen in the Indian population may be biologically different from that encountered in Western practice. Similarly a higher Her-2/Neu immunoreactivity may be inherent in breast tumours in Indian women. Numerical chromosomal abnormalities supplemented by the traditional histological parameters and hormone receptor analysis, appears to be useful.

Conflicts of Interest

None identified

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