RESEARCH COMMUNICATION

Estrogen Receptor, Progesterone Receptor, HER2/neu, P53 and Ki-67 status of Male Breast Carcinomas in Pakistan

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Abstract

Objective: The aim of present study was to assess the status of estrogen and progesterone receptors, HER2/neu, p53 and ki-67 in male breast carcinomas in our institute. Study Design: A descriptive study, carried out in the Department of Pathology (Histopathology), Army Medical College, Rawalpindi, Pakistan from June 2008 to January 2009. Subjects and Methods: In this study 45 cases of male breast carcinoma, including all the histological subtypes were assessed with original pathology reports of each case investigated for the age, laterality of breast, histological type of tumour and tumour grade. Tumour blocks of each case were retrieved for immunohistochemical staining of estrogen and progesterone receptors and HER2/neu, ki-67 and p53 scoring was accomplished using established protocols. Results: The majority of the cases were above 65 years of age. Histologically, the invasive ductal carcinoma was the predominant lesion. In total 95.5% of the cases were estrogen and progesterone (ER and PR) receptor positive. The HER2/neu staining was positive in 84.4% cases. According to the percentage of nuclear staining, 77.7% of the cases were p53 positive and 35.6% of the cases had strong nuclear staining intensity. A total of 55.5% of the tumours showed proliferation by ki-67. Conclusion: The majority of male breast carcinomas in Pakistan are ER and PR positive and demonstrate immunoreactions for prognostic markers. The results point to a relatively aggressive nature of such lesions in our institute.

Key Words: Male breast carcinoma -estrogen/progesterone receptors - HER2/neu - P53 - Ki-67 - prognostic markers

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Introduction

Male breast carcinoma is a rare malignant epithelial tumour. It is usually a disease of the elderly and accounts for less than 1% of all cancers in men and less than 1% of all diagnosed breast cancers in the world (Jamal et al., 2006). In different Asian countries the incidence of male breast cancer is relatively high, In Egypt the incidence is 3%, in Iran it is 5.7%, in India 3.2% and in Arab countries the incidence up to 10% is reported (Van et al., 2005).

In a study of our set up previously 3% of all breast malignancies in both sexes (Jamal et al., 1994) and now 5.9% of all breast carcinomas in both genders were reported and this increase in frequency was found to be statistically significant ($p<0.05$) (Jamal et al., 2006). There is a general impression that it is a more aggressive disease compared to its counterpart in females (Ravandi-Kashani et al., 1998). It has poor survival due to diagnostic delays and older age of the patients at the time of presentation, and due to the proximity of male breast cancer to the chest wall, with shorter lymphatics allowing rapid dissemination of tumour (Crichlow, 1972; Quriel et al., 1984).

To find out the aggressiveness of male breast carcinoma various immunohistochemical markers are used as prognostic or predictive markers (Ellis, 2002) and with this fact in mind this study was planned to assess the status of estrogen and progesterone receptors, Her2/neu, p53 and ki-67 of male breast carcinoma in our institute.

Materials and Methods

A total of 45 cases of male breast carcinoma that presented in Army Medical College and Armed Forces Institute of Pathology Rawalpindi, Pakistan were studied according to a set protocol. Tumour blocks from each institution were retrieved for immunohistochemical staining for estrogen and progesterone receptors as well as other prognostic markers as Her2/neu, ki-67 and p53. Original pathology reports were assessed for the name, age, sex, laterality of breast. From each tumour block new slides were prepared. Haematoxylin and eosin staining was done. Immunohistochemical indirect technique was used for detection of estrogen and progesterone receptor, Her2/neu, p53 and ki-67 status using established antigen retrieval method. The H&E slides were examined and the tumour grade and histological type was noted. The immunohistochemical stained slides were evaluated for each prognostic marker. The results were obtained by means of scoring of prognostic markers. Scoring was done by a set protocol for each prognostic marker. Scoring of

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ER and PR was done by H-scoring method (Ellis, 2002). The Her2/neu status of each case was scored by Hercep (DAKO) scoring method (Hung and Lau., 1999). For p53, intensity of the nuclear staining and the percentage of stained cells were evaluated (Kroger et al., 2006). For ki-67, only the percentage of positive nuclear staining in the tumour was determined (Lyzogubov et al., 2005). The data were fed into a computer programme SPSS version 15 for Windows and the results were analysed.

Results

Forty five cases of male breast carcinoma were included in the study, with a median age of 60 years (range; 30-87 years). The maximum number of patients belonged to the age group above 65 years. The disease was found to have more predilections for the left breast. The predominant histological subtype was invasive ductal carcinoma 37 cases (82.2%). Other subtypes were invasive papillary carcinoma, 4 cases and one case each of invasive lobular carcinoma, mucinous carcinoma, mixed invasive ductal and lobular carcinoma, mixed invasive ductal and mucinous carcinoma. The predominant grade was Grade II (60%) followed by grade III (27%) and grade I (13%). The clinical and histological characteristics of the study population are summarized in Table 1.

Out of 45 cases of MBC, 43 (95.5%) were estrogen receptor positive and only 2 (4.4%) cases were estrogen receptor negative. Among the positive cases, 12 (26.7%) cases showed mild positivity (51-100% nuclear staining), 8 (17.8%) cases showed moderate positivity (101-200% nuclear staining) and 23 (51%) cases were strongly positive (>201% nuclear staining). For progesterone receptor, 43 (95.5%) cases were progesterone receptor positive and 2(4.4%) were progesterone receptor negative. Among the positive cases, 8 (17.8%) cases showed mild positivity (51-100% nuclear staining), 8 (17.8%) cases showed moderate positivity (101-200% nuclear staining) and 27 (60%) cases were strongly positive (>201% nuclear staining).

The Her2/neu staining was positive in 38 (84.4%) cases and negative in 7 (15.5%) cases. The intensity of nuclear staining for p53 is shown in Table 2. Among the positive cases, 36 (80%) cases showed strong positivity and the results of ki-67 staining showed 25 (55.5%) positive and 20 (44.4%) negative cases The immunostaining of ER and PR is shown in Figure 1 and for Her2/neu, p53 and ki-67 is shown in Figure 2.

Table 1. Clinical and Histological Characteristics of the Cases (n = 45)

Variable		Number	(%)
Age in years (range)		60	(30-87)
Laterality of breast:	Left	19	(42.2)
	Right	15	(33.3)
Tumor subtypes (carcimomas):			
Invasive ductal (duct)		37	(82.2)
Papillary		4	(8.9)
Invasive lobular (lob)		1	(2.2)
Mucinous (muc)		1	(2.2)
Mixed duct/lob		1	(2.2)
Mixed duct/muc		1	(2.2)
Grades:	Grade 1	6	(13.3)
	Grade 2	27	(60.0)
	Grade 3	12	(26.7)

Table 2. Scoring of p53 Status According to the Intensity of Nuclear Staining (n=45)

Intensity	No. of cases	Percentage
No staining	11	24.4%
Mild	6	13.3%
Moderate	12	26.7%
Strong	16	35.6%

Discussion

Male breast carcinoma is a rare malignant epithelial tumour, especially in relation to its counterpart in females (Jamal et al., 1994). The incidence of male breast carcinoma in the developing countries has increased significantly over the last two decades. It is comparatively higher in Pakistan and underdeveloped countries, particularly African and Asian countries (Jamal et al., 2006; Akosa et al., 1999). The predominant histological subtype of invasive male breast carcinomas in the present study was invasive ductal carcinoma and same was observed previously in Pakistan (Jamal et al., 2006; Jamal et al., 1994) and abroad (Rodriguez et al., 2002).

There is a general impression that it is an aggressive disease compared to female breast carcinomas (Ravandi-Kashani et al., 1998). To find out the aggressiveness of MBC various immunohistochemical markers are being used as prognostic or predictive markers. Their expression

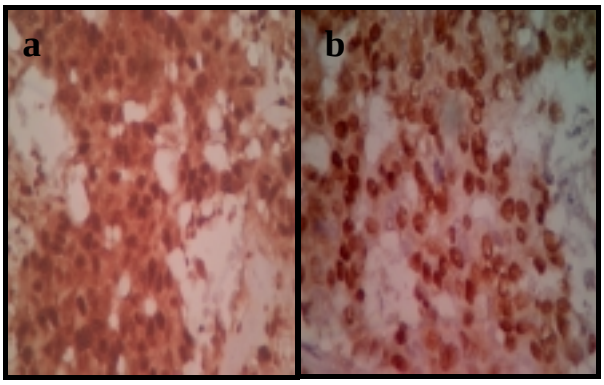

Figure 1. Receptors in an Invasive Ductal Carcinoma. Note strong ER immunostaining in "a" and PR immunostaining in "b" (IHC x 420).

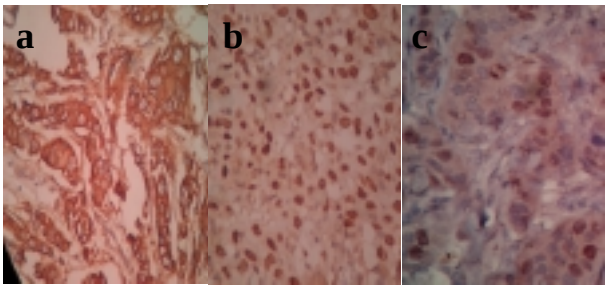

Figure 2. Markers in Invasive Ductal Carcinomas. Note strong (3+) HER2/neu immunostaining in "a", strong (score 4) p53 immunostaining in "b" and strong ki-67 immunostaining in "c" (IHC x 420).

helps in knowing the prognosis of the disease and to accordingly plan the treatment. Higher percentage (95%) of estrogen and progesterone positivity in MBC has been reported (Rodriguez et al., 2002) and same was observed in the present study. One large population-based study of hormone receptors in male breast cancer found a marginally significant trend for patients with ER-positive tumours to present with higher stage disease (Rayson et al., 1998). Many studies have confirmed that male breast cancer has a higher proportion of ER positivity than female breast cancer (Ravvandi-Kashani et al., 1998; Clark et al., 2000; Munoz et al., 1998). It is said that this finding does not correlate with a better prognosis, as it does in women (Munoz et al., 1998).

The Her2/neu is overexpressed in 9% to 45% of female breast cancers, while in men overexpression of up to 95% has been reported (Pich et al., 1999). Expression of Her2/neu may have questionable prognostic significance in male breast cancer. Studies have associated Her2/neu expression with decreased overall survival (Pich et al., 2000). Studies have also shown that in MBC over-expression of Her2/neu correlates significantly with probability of relapse, increased staging, and higher grades of the carcinoma (Bloom et al., 2001). In a study of Rodriguez et al (2002) Her2/neu was associated with shorter disease free survival and correlated with positive lymph nodes. Yamashita et al., (2004) showed that over-expression of Her2/neu was associated with poor prognosis. In the present study also majority of the MBC cases were Her2/neu positive indicating the aggressiveness and poor prognosis.

In the present study, according to the percentage of nuclear staining majority of the cases were p53 positive and majority showed strong intensity of staining (score 3). In another study by Kröger et al., (2006) 70% positivity for p53 accumulation was reported. Between 18 and 58% of MBC were positive for p53 in some other series (Anelli et al., 1995; Willsher et al., 1997), suggesting that MBC contained abnormally expressed, mutant p53 protein. These studies showed a trend toward poorer outcome and larger tumour size. Over expression of p53 has been correlated with recurrence and poorer prognosis in some patients (Rodriguez et al., 2002). In the present study there was over-expression of p53 in majority of MBC cases, indicating the poor prognosis.

The Ki-67(MiB1), a measurement of cell proliferative activity, was identified as a potential prognostic marker in breast carcinoma in which a higher percentage correlated with increased tumour mitotic index and tumour grade (Weidner et al., 1994). Previous studies showed that 20-40% of male breast carcinomas were positive for ki-67 and these carcinomas had weak to strong associations between high ki-67 scores and worse prognosis (Willsher et al., 1997; Margaria et al., 1999). In the present study ki-67 was positive in 56% cases. It was shown by Cozzaniga et al., (2006), that ki-67 expression was seen in 91% of the samples that were positive for atypical cells. This fact might represent that ki-67 may be used in future as a surrogate biomarker in early phases of chemoprevention trials. Tumour expression of ki-67 has also shown to be good predictor of lymph node metastasis in MBC (Anderson et al., 2002).

In conclusion, the higher percentages of ER, PR and proliferative markers positivity in this study show that male breast carcinomas are probably more aggressive and overall survival rates for male breast carcinoma are lower. The data of the present study may have practical implications for the management and treatment strategies of male breast carcinoma. Larger clinical studies and studies of pathological markers to find out overall survival would be helpful so that the exact correlation of marker's positivity with prognosis of the disease can be established. Certain impediments like inability to perform randomized trials in male breast cancer due to overall low incidence of the disease should prompt efforts at setting up large multi-institutional, worldwide studies, so data could be shared and pooled together as a data base to enable emergence of meaningful therapies to treat and improve survival.

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