

Original Article

Male Breast Carcinoma: An Immunohistochemical Study of 50 Cases From Iran

Farrokh Tirgari¹, Afshin Abdi Rad¹, Fatemeh Mahjoub¹,
Mahmoud Mohammadi¹, Nargess Tabarzan¹, Omid Emadian¹

1. Dept. of Pathology and Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

Background and Objective: Male breast carcinoma (MBC) is an unusual form of neoplasia, representing 0.7 to 1 percent of all breast cancer cases. Usually, the carcinoma affects patients after the sixth decade. The aim of this study was to evaluate the status of estrogen and progesterone receptors (ER and PR) and prognostic factors (p53 and Her-2/neu) in a series of male patients with breast cancer and correlate them with tumor grade and stage.

Materials and Methods: Fifty cases of breast carcinoma in male patients, retrieved from the files of the Cancer Institute from 1996 until 2005 were included in this study.

Results: Most of the cases were categorized as grade 2 (65.3%), grade 1 cases comprised 20.4% and grade 3 was 14.3%. Stage IIb were the largest group (32%).

Estrogen receptor was detected in 90% of cases and progesterone receptor in 68% of cases and no significant correlation was found between estrogen and progesterone receptor positivity and tumor grade or stage. In addition, p53 and Her-2/ neu staining revealed positivity in 11 cases (27.5%) and 13 cases (26%) respectively with strong positivity in only 6 cases and no significant correlation was found between tumor grade and stage and p53 expression. It is clear from our data that Her-2/neu positivity in MBC is lower than in female breast carcinoma.

Conclusion: This study, which comprises rather large series of MBC in Iran during a 10-year period, shows that most patients refer in rather late stages and prognostic factors such as p53 and Her-2/neu has no significant correlation with tumor grade and stage at presentation in our patients.

Key Words: Breast cancer, Male, Pathology, Prognosis

Introduction

Male breast carcinoma (MBC) is an unusual form of neoplasia, representing 0.7 to 1 percent of all breast cancer cases, although it is higher in other countries like Egypt in which the incidence rises to nearly 10%

(1). Increased incidence of breast carcinoma is seen in patients with Klinefelter's syndrome and it seems to be a pathogenic relationship between gynecomastia and carcinoma (1). In a study in Turkey it was demonstrated that a close relationship exists between exposure to

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Address communications to: Dr. Fatemeh Mahjoub, Cancer Institute, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran.

Email: fmahjoub@sina.tums.ac.ir

electromagnetic fields and light at night in male breast carcinoma in eastern Turkey (2). 14 out of 1000 cases are bilateral (3). Usually, the carcinoma affects patients after the sixth decade, with a mean of 59 years of age (3). From a pathologic viewpoint, carcinoma of the male breast is not very different from that of the female, the most common histological type (84 percent) being the infiltrating ductal carcinoma (3,4). Papillary intraductal (5 percent), medullary (4 percent), and mucinous (1 percent) carcinoma have been reported in the literature, whereas infiltrating lobular carcinoma is seen only in patients with Klinefelter syndrome. The neoplastic cells are hormone dependent, as are the female breast malignancies (3) and the incidence of positivity for estrogen receptors (ER) is higher than in females (1) and is stated to be 80% to 93% (4,5) in contrast to 55% to 65% positive expression in female breast carcinoma (5,6). Few investigations have been done on the level of progesterone and estrogen receptors in gynecomastia and revealed that absence of elevated estrogen and progesterone receptors in patients with idiopathic gynecomastia may serve to clarify why these patients' disease rarely progress into malignancy (7). The incidence of axillary's metastasis is the same in men as in women but the prognosis is slightly worse in males especially for stage II and III disease (1,8). Compared with cases diagnosed in females, male breast malignancies are usually in a more advanced stage (3,4), in addition, the male patients are usually older than females when diagnosis is made. Consequently, the prognosis is strictly related to the time of diagnosis. Because the mammary gland in males is small, with a very rudimentary structure, invasion of the lymphatic vessels, soft tissue, and pectoralis fascia will be rapid (3,9). The primary treatment for male breast carcinoma has long been radical mastectomy. More recently, however, radical modified mastectomy with node dissection or simple mastectomy followed by postoperative chemotherapy and radiotherapy have become treatments of choice (3). Several tissue prognostic factors are suggested by several authors such as apolipoprotein D (Apo D) which is a protein component of the human plasma lipid transport system and is present in benign and malignant human breast tissues with positive association of Apo D content in male breast tumors with favorable outcome (10), lysozyme which is a major protein component of human milk and analysis of relapse-free survival (RFS) showed that high lysozyme values were significantly associated with shorter RFS periods (11). Also expression of prostatic

specific antibody is investigated by some authors that found no PSA reactivity in male breast carcinoma but normal and hyperplastic duct epithelium in gynecomastia exhibited focal strong reactivity (12). Prognostic markers such as c-myc, c-erbB-2, p53, and bcl-2 proteins have been investigated by some authors (13,14) and over-expression of c-myc, c-erbB-2, and p53 proteins may be regarded as additional worse prognostic factors (13). Few studies have shown that ERBB2 over-expression and/or amplification can be detected in male breast carcinoma, yet at lower frequency (~10%) than in female breast carcinoma (5,6) and have shown that the presence of ERBB2 gene abnormalities which is located on chromosome 17, together with high pathologic stage are significantly correlated with poor survival but no correlation was observed between chromosome 17 aneuploidy and overall survival (5).

The aim of this study was to evaluate the status of estrogen and progesterone receptors and also prognostic factors p53 and Her-2/neu using immunohistochemical techniques in a series of 50 male patients with breast cancer and to correlate them with tumor grade and stage.

Materials and Methods

Fifty cases of breast carcinoma in male patients, retrieved from the files of the Cancer Institute, affiliated with Tehran University of Medical Sciences, Iran, from 1996 until 2005 were included in this study. Histological type was evaluated according to the WHO classification. Tumor differentiation was assessed using the Nottingham modification of the Bloom Richardson grading system (1) and pathologic stage by the TNM-UICC system (15). Formalin-fixed, paraffin-embedded tumor biopsies were retrieved from the archives and a representative tissue block of each case selected for immunohistochemistry (IHC). From each block, 2 to 3 μ m-thick sections were cut and mounted on coated slides, and stained by the biotin-streptavidin-peroxidase method using the antibodies against estrogen and progesterone receptors, p53 and Her-2/neu (antibody informations are depicted in Table 1). The slides were incubated for 30 minutes at room temperature for Her-2/neu and one hour for others after epitope exposure in a microwave oven with 0.01 M sodium citrate buffer, pH 6. Endogenous peroxidase was blocked using 3% hydrogen peroxide. Specific staining of these markers (nuclear and cell membrane antigens respectively) was evaluated

semi-quantitatively by 2 investigators (F. Tirgari, and A. Abdi Rad). Tumors were considered positive for ER and PR whenever the percentage of tumor cells with brown stained nucleus was higher than 5%. The presence of cytoplasmatic staining was regarded as nonspecific. Her-2/ neu expression was considered positive when complete membrane staining was present in more than 10% of cells. Positive staining was scored as moderate (2+) or intense (3+). p53 expression was considered positive when more than

10% of tumor nuclei stained positive. The following subgroups were created for each parameter to assess its individual prognostic value: pathologic stage (stage I vs. stage II vs. stages III and IV), grade of differentiation (G1 vs. G2 vs. G3), ER (negative vs. positive), PR (negative vs. positive), p53 (positive vs. negative) and Her-2/neu expression (score 0 and 1+ vs. scores 2+ and 3+) and data were analyzed using Chi square analytical test using SPSS version 14.1. A p value less than 0.05 was considered as significant.

Table 1: Information of antibodies used in this study

	Company	Product Code	Clone	Dilution	Buffer
ER	DAKO (Denmark)	M7047	Clone 1D5	1:50	0.01 M sodium citrate
PR	DAKO (Denmark)	M3569	Clone PgR 636	1:80	0.01 M sodium citrate
Her-2/neu	DAKO (Denmark)	A0485	Polyclonal	1:200	0.01 M sodium citrate
p53	DAKO (Denmark)	M7001	Clone DO-7	1:250	0.01 M sodium citrate

Results

In this study, 50 male patients were included in the study; age range was 27 to 82 years and mean age was 58.88 years. Forty four cases (88%) had invasive ductal carcinoma. Other variants included invasive papillary carcinoma (4%), invasive lobular carcinoma (2%), invasive cribriform carcinoma (2%), mucinous carcinoma (2%) and ductal carcinoma in situ (2%). Vascular invasion was detected in 32% and perineurial invasion in 22% out of them. Four cases were multifocal and in situ component was present in 6 cases of invasive ductal carcinoma. Most of the cases were categorized as grade 2 (65.3%), grade 1 cases comprised 20.4% and grade 3 were 14.3%. Staging is depicted in Table 2 with stage IIb as the largest group (32%).

Estrogen receptor was detected in 90% of cases (45 cases) (Figure 1) and no significant correlation was found between estrogen receptor positivity and tumor grade or stage.

Table 2: Number of patients and percent of different stages of breast carcinoma

Stage	Number of patient	Percent
I	7	14
IIa	13	26
IIb	16	32
IIIa	2	4
IIIb	10	20
IV	2	4
Total	50	100

Progesterone receptor was positive in 68% of cases (34 cases) and again there was no significant correlation between positivity of receptor and tumor grade and stage.

Meanwhile, p53 and Her-2/ neu staining revealed positivity in 11 (27.5%) and 13 cases (26%) (Figure 2) respectively, with strong positivity in only six cases

and no significant correlation was found between tumor grade and stage and p53 expression

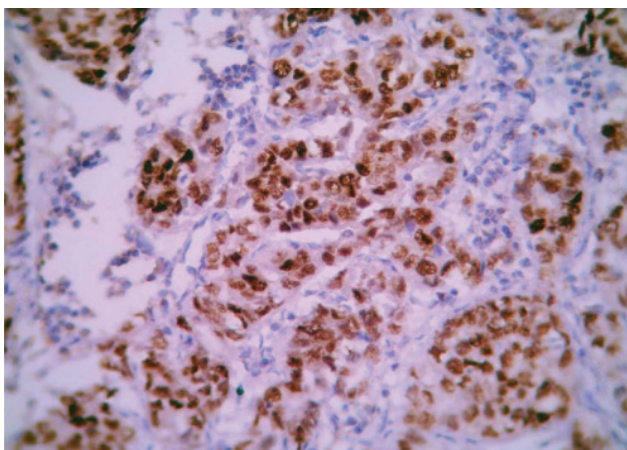


Figure 1. Immunohistochemical staining using avidin-biotin method for estrogen receptor showing strong nuclear positivity in most cells ($\times 400$)

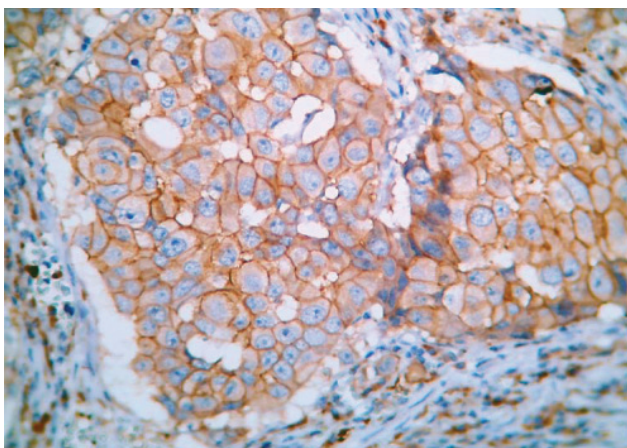


Figure 2. Immunohistochemical staining using avidin-biotin method for Her-2/neu oncogene showing strong membrane positivity in most cells ($\times 400$)

Discussion

In this study, ER, PR, p53 and Her-2/neu were investigated in a series of 50 archival cases of male breast carcinoma.

Histologically, most of our cases were of invasive ductal type (88%) and other subtypes were seen with much lower frequency (2% each), the same reported by most authors (1,4,5). Most of the MBC in our series were moderately differentiated (G2) tumors (65.3%) which is consistent with other studies (5), although some studies found a slight predominance of poorly differentiated (G3) versus well-differentiated (G1)

tumors (6,16) whereas others found the reverse (17). Most of our cases referred in stages II and III, the same as in other studies (5), which is rather late for this type of tumor. The commonly referred statement that the positivity of ER is high in male breast carcinoma (1,4-6) was confirmed in our study as 90% of cases were positive for this receptor and it did not have any correlation with tumor grade and stage at diagnosis. In other studies, it has been stated that hormone receptors status showed no impact on overall survival (18). On the contrary, significant correlation was seen between the pathologic stage of disease and overall survival rate by most authors (5), prognosis being more favorable for cases treated at early stages.

The most frequently used technique for assessing Her-2/neu expression is IHC, which detects cases with over-expression (2+ and 3+). In our series, the incidence of Her-2/neu protein over-expression was 26% and we found no correlation between positivity of this marker and tumor grade and stage, the same reported by some authors (19). Prior studies in which immunohistochemical analysis of Her-2/neu in MBC was assessed (5,16,20) showed positive results varying from 2% to 56%. These discrepancies in IHC-based studies are probably related to technical and interpretation variability, different scoring systems and cut-off values.

It is clear from our data that Her-2/neu positivity in MBC is lower than in FBC. One may speculate that this fact could be related to a competition between estrogen/progesterone (known to be positive in a higher percentage in MBC than in FBC) and other co-regulatory receptor proteins resulting in altered Her-2/neu expression (21). Positivity of p53 was 27.5% in our series with only 6 patients showing strong positivity (3+) and did not have any correlation with tumor grade and stage. Positivity of p53 varies among different studies from 2 to 63% and prognostic value is also debated by most authors (13,14).

Conclusion

This study which comprises rather large series of MBC in Iran during a 10-year period shows that most patients refer in rather late stages and prognostic factors such as p53 and Her-2/neu have no significant correlation with tumor grade and stage at presentation in our patients.

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