Histological and Immuno-histochemical Study of Male Breast Carcinoma in Northern Pakistan

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Abstract

Objective: To study routine histological as well as immunohistochemical prognostic markers including ER, PR, p-53 and Her-2/neu in male breast carcinoma and compare the above parameters with breast carcinoma in female patients of the same population. Association of the immunohistochemical markers with histological prognostic markers was also studied.

Methods: Cross-sectional descriptive study was carried out from January 2004 to May 2007 at AFIP, Rawalpindi on 55 cases of male breast carcinoma. Assessment of tumour size on gross examination followed by microscopic evaluation of tumour grade and lymph node metastasis was done. Immunohistochemistry was performed for ER, PR, Her-2/neu and p-53.

Results: The male breast cancer patients had a mean age at diagnosis of 57 years with a mean tumour size of 4.25cm, constituting 4.7% of malignancies in men. Infiltrating ductal carcinoma was the predominant subtype with 47(87.3%) cases. Majority of the tumours were grade II (64.2%) followed by grade III (32.1%). Out of 28 cases with axillary clearance; lymph node metastasis was present in 20 (71%) cases. ER and PR expression was seen in 81% and 79.3% cases respectively while Her-2/neu expression was seen in 24.5%. p-53 was expressed in 14(26.4%) patients. No association of immunohistochemical markers was seen with lymph node and tumour grade.

Conclusion: Male breast cancer has a higher incidence and occurs in a decade younger than the Western population with a larger tumour size and higher number of patients with lymph node metastasis. Men have a higher ER, PR expression as compared to female breast cancer patients without any association with histological prognostic markers. Her-2/neu and p-53 expression in males is comparable to the West but their prognostic role in terms of survival and treatment strategies needs to be ascertained by follow up studies (JPMA 59:67; 2009).

Introduction

Carcinoma of the male breast is a disease with limited available information in terms of biology, natural history and treatment strategies. Establishment of risk factors is a grey area due to rarity of the disease. However raised estrogen levels, testicular disorders, gynaecomastia and dietary factors are amongst the most frequently studied epidemiological risk factors.[1] BRCA-2 gene and XXY karyotype have also been implicated in its aetiology.[2]

Western literature reports the incidence of male breast cancer to be <1% of all carcinomas.[1] The mean age at the diagnosis in men is 68 years, which is 10 years more than that for breast carcinoma in females.[3] On the contrary, Asian,[4,5] studies report a much higher incidence and much younger age which suggests different tumour biology or risk factors in this population. However limited data in terms of histological prognostic markers, hormone receptor expression and survival studies are available from these developing countries.

The objectives of this study were to determine the frequency of histological prognostic markers, hormone receptor expression for estrogen (ER) and progesterone (PR) and mutated p-53 in carcinoma of the male breast in our population Moreover frequency of Her-2/neu expression which has a therapeutic and prognostic role in female breast carcinoma was also evaluated to suggest its role in male breast carcinoma. The expression of the above parameters was compared with that of female breast carcinoma in the same population. Association of the hormone receptors, biological factors and mutated p-53 with histological prognostic markers was also assessed.

Materials and Methods

This study was carried out at Armed Forces Institute of Pathology (AFIP), Rawalpindi from January 2004 to May 2007. AFIP is a referral laboratory with an annual histopathology workload of approximately 26,000 surgical biopsy specimens. It receives samples from armed forces hospital establishments, Northern Punjab, North West Frontier Province and from adjoining civil and private hospitals of Rawalpindi and Islamabad region.

All the cases of male breast carcinoma diagnosed on biopsy, lumpectomy or mastectomy with or without axillary clearance were included in the study. Measurement of the tumour size was done on gross examination in cases of lumpectomy and mastectomy. Representative sections of the tumour and lymph nodes were processed for paraffin embedding after formalin fixation. 3-4 μm thick sections of the tissue were made and stained with haematoxylin and eosin (H&E) for subsequent microscopy. Histological type of the tumour, lymph node metastasis and confirmation of tumour size was done on routine H&E sections. Histological tumour grading was done using Modified Bloom Richardson scoring system.[9]

Immunohistochemical determination of ER, PR, Her-2/neu and p-53 was done with monoclonal antibodies using antigen-antibody Strept-avidin immunoperoxidase technique. ER and PR positivity was assessed using H-scoring system taking nuclear staining with a score >50 as positive.[10] Her-2/neu immunohistochemical expression was assessed using DAKO scoring system taking only strong membrane staining with a score of 3+ as positive.[11] p-53 mutation was assessed immunohistochemically taking strong nuclear staining as positive.

Data was entered in SPSS version 11.0 and statistical analysis was done to determine frequency and Chi square test and Fischer's exact test to determine association amongst immunohistochemical markers and prognostic markers.

Results

During the study period from January 2004 to May 2007, a total of 9432 malignant tumours were diagnosed in both males and female patients. There were 1195 cases of breast carcinoma constituting 12.7% of all the malignancies. Amongst them 55 (4.6%) were male breast carcinomas with a male to female ratio of 1:21. Breast carcinoma constituted 1% of 5546 malignant tumours in males and <1% of all malignancies diagnosed during this period.

The mean age at diagnosis was 57±16 years with a range of 15-85 years. Maximum numbers of cases were seen in the fifth and sixth decade. The different types of specimens in the cohort consisted of 12 biopsies, 13 lumpectomies and 30 mastectomies. The mean tumour size was 4.25 cm with a range of 1.5-8 cm. Majority of the tumours were larger than 2 cm and only 4(9.8%) cases out of 43 had a tumour size <2cm.

On histology, infiltrating ductal carcinoma (IDC) was the predominant subtype comprising 48 (87.3%) cases. There were 2 (5.5%) cases of infiltrating lobular carcinoma which were negative for E-cadherin on immunohistochemistry. One case each of invasive papillary carcinoma and mucinous carcinoma was also diagnosed in elderly males in 8th decade. A single case of adenoid cystic carcinoma was also diagnosed in a 15 years old adolescent male.

Using Modified Bloom Richardson scoring system, 34 (64.1%) carcinomas were histlogically graded as grade II, 17 (32.1%) cases were grade III and 2 (3.8%) were grade I carcinomas.

Axillary lymph node dissection was done in 28 out of

30 mastectomies. There were 20(71%) cases with metastasis in the axillary lymph nodes. The average number of lymph nodes involved by metastasis was 4 with 24 lymph nodes being the highest number of involved nodes in any one case.

Hormone receptor expression for estrogen was seen in 43 (81.2%) cases and positivity for progesterone receptors was present in 42 (79.3%) cases. Her-2/neu expression using DAKO score showed 35 (66%) tumours to have 0 /1+ score while 5 (9.5%) cases had a score of 2+. Intense complete membrane staining with DAKO score 3+ was seen in 13(24.5%) cases considered as positive out of the 53 cases.

Mutated p-53 expression was seen in 14(26.4%) cases in the form of specific intense nuclear staining.

Comparison of the frequency of histological prognostic markers, hormone receptor status and Her-2/neu expression amongst male and female carcinomas[6] of the Pakistani population are summarized in Table 1.

No association of ER, PR, Her-2/neu and p-53 was seen with tumour grade and lymph node metastasis (Table 2).

Table 1: Comparison of tumour characteristics of male and female breast carcinoma.

Prognostic parameter		Male Breast Cancer (n=55) %	Female Breast Cancer (n=100) %
Histological type	Infiltrating ductal carcinoma	87.3	91
	Infiltrating lobular carcinoma	5.5	3
	Others	7.2	6
Histological grade	Grade I	3.8	6
	Grade II	64.1	68
	Grade III	32.1	24
Tumour size	<2cm	20.9	17
	2.1-5cm	55.8	56
	>5cm	23.3	27
Lymph node metastasis	Present	69	65
	Absent	31	35
Age range in years		15-85	22-86
Age	7th decade	17	14
	6th decade	11.3	18
	5th decade	26.4	35
	4th decade	20.8	20
	3rd decade	15.1	11
ER	Positive	81.2	74
	Negative	18.8	26
PR	Positive	79.3	72
	Negative	20.7	28
Her-2/neu	Positive	24.5	29
	Negative	75.5	71

Discussion

Male breast carcinoma presents either as a painless firm sub-areolar mass or a mass in the upper outer quadrant of the breast.[3] Men are more likely to have a delay between the onset of symptoms and diagnosis of carcinoma because of limited awareness of breast carcinoma in males. This delay is responsible for the higher stage of presentation in men as compared to women in the West.[1] However in Pakistan, women also present with cancer at a higher stage due lack of awareness and social inhibitions.[7] Therefore relatively later stage of presentation in men with breast cancer seen in the West is not the case in our population.

Cancer of the male breast behaves like female breast carcinoma following similar patterns of local invasion and metastasis.[3] Almost all the histological types of breast carcinomas in women have also been reported in men.[1] Invasive ductal carcinoma (IDC) is the commonest type constituting up to 80% as reported in the western literature.[8] In our study it constituted 87% of cases. A higher incidence of more than 90% cases of IDC has been documented in local studies previously.[5] ILC is reportedly much less common than women and accounts for up to 1% cases[9] while the present study showed 3(5.5%) cases. Rare subtypes like mucinous carcinoma, invasive papillary carcinoma, tubular carcinoma have been reported but are uncommon with their percentage depending upon the sample size of the study cohort.[10,11]

Higher tumour grade is associated with a poor prognosis and a significant difference in 5-years survival.[12] Moreover higher tumour grade is also associated with a higher number of lymph nodes involved by tumour at the

Table 2: Association of ER , PR , Her-2/neu and p53 expression with lymph node metastases and tumour grade in male breast carcinoma.

Prognostic markers		ER Positive	ER Negative	p- value	PR Positive	PR Negative	p- value	Her-2/neu Positive	Her-2/neu Negative	p-vlaue	p-53 Positive	p-53 Negative	p- value
Tumour grade (n=53)	**Grade I**	2	0	0.78	2	0	0.71	0	2	0.45	0	2	0.16
	Grade II	27	7		26	8		6	28		5	29	
	Grade III	14	3		14	3		7	10		9	8	
Lymph node mets (n=28)	**Present**	14	6	0.33	13	13	0.23	5	15	1.0	7	11	0.52
	Absent	7	1		7	7		2	6		2	8	

time of surgery.[3] Since majority of our tumours were either in grade II (64%) or III (32%), this explains the 69% lymph node metastasis in our patients. Local studies by Kiyani[13] and Jamal et al[14] also show a predominance of grade II and III tumours. However, Muir et al in their study of 75 cases reported a very high percentage (85%) of grade III tumours.[15] At the same time, grade II and III tumours constituted the highest number of male breast cancers in the study by Wang-Rodriguez et al with equal proportion of cases with or without lymph node metastasis which shows a relatively early diagnosis and better prognosis in terms of survival.[3]

Tumour size is a significant prognostic marker in male breast cancer.[16] Higher survival rate up to 85% is seen with tumours <2cm in size[17] but in the present study only 7% cases had a size <2cm indicating higher tumour stage at diagnosis. An earlier study by Jamal et al[14] has also shown a higher tumour size at presentation but variable results are seen in the West with some showing a smaller tumour size at presentation[3] while larger tumour size with advanced stage[15] is seen in others.

Attempts to study male breast carcinoma at molecular and genetic level have been limited by small number of patients and lack of follow up data. ER and PR expression in female breast cancer correlates with survival and response to estrogen antagonists. Higher proportion of positivity is seen in male breast cancer as compared to female breast cancers but this does not correlate with better prognosis as in women.[18] Hormone expression in our study of male breast cancer patients in comparison to female breast cancer was slightly higher for ER (81% vs 74%) and PR (79% vs 72%) and similarly reported by other studies[19,20] as well. ER and PR expression in our study is comparable to 81% and 71.1% positivity for ER and PR respectively reported by Muir et al.[15] However hormone expression up to 95% has also been mentioned by Wang-Rodriguez et al.[3] ER positive tumours had 66.6% lymph node metastasis in the largest population based study of male breast cancer showing a trend for patients with ER expression to have a higher stage disease.[21] Aberrant steroid receptor up-regulation as a consequence of constitutive activation of downstream targets in the estrogen starved post-menopausal setting and in male breast patients have been postulated for hormone receptor expression.[15]

p-53 gene mutations are very frequently observed (%?) ref (?) in female breast cancer. p-53 inhibits cell death through apoptosis and the mutant p-53 is detectable by immunohistochemical expression.[3] Mutant p-53 is associated with poor prognosis in female breast cancer. Similarly poor prognosis has also been reported in male breast carcinomas.[15] Some researchers[22,23] have shown an expression between 18-58% while 26% expression was seen in the present study. No correlation of p-53 has been seen with lymph node metastasis and grade in our study which is also seconded by absence of correlation between p-53 expression and prognosis in male breast cancer in the study by Willsher et al.[23]

C-erbB-2 also known as Her-2/neu is a proto-oncogene that encodes for transmembrane receptor of the tyrosine kinase family. It is expressed in 21-30% of female breast cancers and is associated with a poor prognosis but entitles the patient to targeted therapy with trastuzumab (Herceptin).[3] Limited data is available with variable expression of Her-2/neu in 1.7-45% in male breast cancer patients.[23,24] Muir et al[15] have reported Her-2/neu expression of 5.3% while Wang-Rondriguez et al[3] showed 9% expression in their study. Present study showed 24% Her-2/neu expression using only DAKO 3+ cases as positive and taking DAKO 2+ as negative. Stringent criteria for evaluation have been suggested as overestimation in the absence of standardization for Her-2/neu may result in erroneous interpretation.[1] Over-amplification of Her-2/neu by FISH was found absent in a study of 58 Her-2/neu positive cases suggesting protein expression without gene amplification in male breast carcinomas.[25] No association of Her-2/neu expression with other prognostic markers is seen.[15] Therapeutic role of Herceptin in male breast cancer still needs validation and trials.

To summarise male breast cancer occurs in a decade younger than the Western population with a larger tumour size and higher number of patients with lymph node metastasis at presentation. Males have a higher ER, PR expression than female breast cancer patients without any association with histological prognostic markers. Her-2/neu and p-53 expression in males is comparable to that seen by other workers but their prognostic role in terms of survival and treatment strategies needs to be ascertained by follow up and further studies.

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