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Metaplastic Breast Cancer and p16 Positivity: What Does It Mean?

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Abstract

Background: Metaplastic breast cancer (MBC) is a heterogeneous group of malignant breast tumor having different morphologic subtypes. Very little is known about the mechanisms which underlie the pathophysiology of metaplastic breast cancer. This study aimed to assess the p16 positivity in MBC cases.

Methods: A single center series of MBC cases were subjected to immunohistochemical (IHC) testing for p16 using a monoclonal antibody.

Results: 14 out of 15 cases showed squamous metaplasia and 13of the latter had p16 positivity in the metaplastic component.

Conclusion: The significance of this high level of positivity of the p16 is curious and its significance if validated in larger numbers is so far uncertain. We present some views on it.

Keywords: Metaplastic; Breast Cancer; p16

Introduction

Metaplastic breast cancer is a heterogeneous group of malignant breast tumors having different morphologic subtypes. Considerable variations exist in different ethnic groups [1].The incidence of metaplastic breast cancer is reported to be 0.2-1 percent of breast cancer and it is rare in the west [2]. MBC is referred to tumor when conventional breast carcinoma contains a metaplastic component ranging from <10% - >50% [3,4].

It is generally felt to be a more aggressive tumor with a greater propensity to have a worse outcome. Very little is known about the mechanisms which underlie the pathophysiology of metaplastic breast cancer [5]. Many of these tumors are triple negative, locally invasive with large tumor size and less likely to involve the lymph nodes [1,5]. Prognostic variables which are normally felt to be so in breast cancer may not be so in MBC. The patterns of failure are early [6]. MBC is treated as conventional breast cancer without regard to the accompanying histology. The best methods of treatment are not known [7]. Most of the data derived is from case series or population based studies.

Recent research has revealed some potentially actionable genetic changes in a subset of these rare tumors. However, ongoing efforts to further characterize the genetic basis and the molecular alterations underlying the distinctive morphological and clinical characteristics of these tumors are needed in order to identify new targets for treatment [8].

Given that squamous cancers are seen with HPV related tumors such as cervical, oropharyngeal, anal and penile the question of breast cancer being a part of this spectrum needs further study as well. The presence of p16 positivity by immunohistochemistry as a surrogate marker in human papilloma virus (HPV) related oropharyngeal cancer is now well accepted [9]. Whether this is related to other such cancers of the HPV spectrum needs more research.

p16 acts as a tumor suppressor gene, a member of INK4 family of CDK inhibitors, inhibits CDk4 and CDK6, maintaining retinoblastoma gene in hypophosphorylated state, thereby preventing G1 to S phase progression of cell cycle. Normal proliferative cells do not express significant level for p16 [10].

The aim of this study was to determine the p16 positivity in metaplastic breast cancer. To the best of our knowledge this is the first study addressing p16 positivity in metaplastic breast cancer. The study was approved by the Departmental research committee and Ethics research committee (reference number ZH/EC-006)

Methods

This prospective study was conducted at department of oncology (section of Breast surgery) at The Ziauddin University Hospital, Karachi, Pakistan. We included patients of MBC diagnosed and treated from January 2014 to June 2015.

Informed consents were obtained from the patients prior to participation in the study. The study was approved by local ethical committee of the Ziauddin University Hospital. The Patients who were lost to follow up during treatment were excluded from the study.

Metaplastic breast cancer was defined by the presence of non-glandular epithelial (squamous) or mesenchymal (spindle or matrix producing) elements >10% associated with invasive mammary carcinoma, cytokeratin 5/6 immuno staining was done also to label the case as MBC. The estrogen receptor (ER), Progesterone receptor (PR), Her 2 neu status and Ki 67 index was determined using the criteria of the College of American Pathologist (CAP).

IHC for p16 was performed using the monoclonal antibody ab 108349(Abcam Ltd). p16 staining was scored on a scale of 0-3 based on the extent of immune positive cell: 0- no staining; 1-<25%(weak); 2-25%-75%(moderate); 3->75%(strong). Both nuclear and cytoplasmic pattern of immunostaining were taken as positive.

Data was collected for age ,tumor size, nodal status, grade, stage, type of tumor, type of metaplastic component, ER, PR, Her2 status, Ki 67 index, type of surgery, adjuvant therapy(chemotherapy, radiation, hormonal), neo adjuvant chemotherapy and p16 positivity. The data was entered on SPSS version 19.0 and analyzed for descriptive statistics like age, grade, percentage of p16 positivity etc.

Results

Characteristics of the cases of MBC are summarized in Table 1 and II:

		Metaplastic Breast Cancer
		N (%)
Total		15 (100)
Age(years)	Mean	50.14
	minimum	30
	maximum	74
Stage(p)		
	1	00 (00)
	2	09 (60.0)
	3	05 (33.4)
	4	01 (6.6)
Neoadjuvant chemotherapy		
		02 (13.3)
Adjuvant chemotherapy		13 (86.7)
Trastuzumab		01 (6.6)
Surgery	BCT	04 (26.7)
	Mastectomy	11 (73.3)
DXRT	Yes	13 (86.7)
	No	02 (13.3)
Adjuvant Hormonal	Yes	02 (13.3)
	No	13 (86.7)

Table 1: Demographics of cases having metaplastic breast cancer.

Total 15 cases were subjected to p16 testing. All patients were female having grade III disease. Triple assessment was done in all cases. Histologic diagnosis of metaplastic breast cancer was achieved in all cases on core needle biopsy before surgery. Cases were staged according to American Joint Committee on Cancer (AJCC) version 7 prior to commence treatment.

Neo adjuvant chemotherapy was given in 2 cases, it was used in one case to down stage the disease however she progressed on treatment while in other case it was given to down size the tumor for Breast conservation having good response. The average tumor size was 3.5 cm (3-6.5). Axillary clearance was done in 11 cases. The median number of lymph nodes involved was 4 (minimum 1-maximum 13). The median number of lymph nodes showing metastatic squamous metaplastic component was 2. One case of MBC (squamous metaplasia) having stage IV disease showed visceral metastasis (Table 2).

Total		**MBC** N (%)
		15 (100)
Histological type	Invasive Ductal	15 (100)
Grade	III	15 (100)
ER/PR	-	13 (86.7)
	+	02 (13.3)
Her-2/neu	+	01 (6.6)
	-	14 (93.4)
Triple negative	Yes	12 (80.0)
	No	03 (20.0)
Type of Metaplasia	Squamoid	14 (93.4)
	Matrix Producing	01 (6.6)
Ki 67	3+	12 (80.0)
	4+	03 (20.0)
p16	+	13 (86.7)
	-	02 (13.3)

Table 2: Pathological features of cases with metaplastic breast cancer.

Squamous metaplasia was seen in 14 cases of MBC while one case was of matrix producing MBC.

p16 staining was positive in 13/15 cases. One case showed weak nuclear staining (score1) while in 12 cases it was scored 3. The matrix producing MBC was negative for p16 immunostaining (Figure 1).

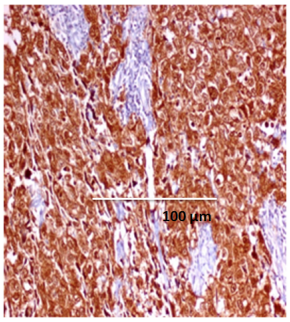

Figure 1: p 16 positivity in squamoid component of metaplastic breast cancer.

Adjuvant chemotherapy was given to 13 cases, standard doxorubicin cyclophosphamide and taxane based regimes were used. Adjuvant Radiation therapy was given to 13 cases according to standard criteria.

Discussion

Metaplastic breast cancer are a group of breast cancer subsets which display the coexistence of breast cancer of the usual varieties along with other components such as squamous, sarcomatoid, chondroid or other tissue types in the same specimen [1]. This is a rare subset in the western women accounting for 0.2-0.6 percent of the breast cancer cases. It is commoner in the African American and Hispanic populations [1,2]. Pakistani women have a high rate of breast cancer which is reported to be the second highest in Asia after Israel. Like elsewhere in the developing world the patients are younger and the disease is aggressive overall [11]. In 1107 cases of triple negative breast cancer reported from Pakistan 10.7% of the cases were metaplastic [12]. Unpublished observations and local experience indicates that more metaplastic breast cancer is being seen locally. It is known that breast cancer may express p16 positivity from 10-55% [10]. There are no data in metaplastic breast cancer for p16 positivity to our knowledge. Metaplastic breast cancer is an aggressive subtype of breast cancer and may be frequently be axillary node negative. According to Hennessy although mostly triple negative they resemble the claudin low subtype, the PIK3kinase pathway is frequently involved and there are stem cell like characteristic and thus there is chemo resistance [13]. A recent abstract at ASCO indicates that with a low RRM1 expression rate, gemcitabine may be a more effective drug than conventional breast cancer chemotherapies [14].

A study conducted prospectively to analyze association between HPV and oropharyngeal cancer showed HPV type 16 as the most frequently encountered type, their result showed statistically significant association between HPV-positive patients and a higher tumor grade [15].

p16 is not a routine marker checked by IHC in breast cancer tissue for clinical purposes. The p16 gene is a tumor suppressor gene located on chromosome 9p21 and is a frequent site of allelic loss in cancers. The cancers include melanomas, esophageal, lung, pancreas, mesothelioma, bladder, head and neck, acute lymphocytic leukemia, brain, osteosarcoma, ovarian, and even breast cancer [10]. The p16 gene is composed of three exons, which encode a 156 amino acid, 15.8 kd protein that blocks progression through the cell cycle by binding to either cyclin-dependent kinase (CDK) 4 or 6 and inhibiting the action of cyclin D. As we noted that squamous metaplastic change was the common subtype as in our cases. We wondered if this change could be due to a HPV infection. Consequently as a first easy step we tested the tumor tissue for p16 by immunohistochemistry which at least in the setting of oropharyngeal cancer is a surrogate marker of HPV infection. The early genes of HPV (of which there are more than a 130 subtypes, some are oncogenic e.g., 16 and 18 subtypes) include the E6 and E7. These neutralize the retinoblastoma protein. As the Rb protein normally inhibits p16 expression this results in p16 overexpression as it is unable to do so [9].

The association of HPV in breast cancer has been noted for many years. However various methodologies have resulted in a very wide spectrum of positivity. In a publication from MexicoRoberto Herrera-Goepfer tested 20 metaplastic breast cancer tissues for HPV. They had 40% positivity. Subset results indicated that there were a total of 10 patients who had squamous elements mixed with carcinoma and or sarcomatoid and chondroid differentiation. Both of the 2 cases which were reported as adenosquamous squamous differentiation were HPV16 positive. Patients reported as carcinoma with squamous differentiation tested negative in four cases and positive in one. Seven cases of squamous with some form of sarcomatous variation were negative for HPV. Interestingly 3 cases with squamous and chondroid differentiation were positive for HPV16 and three were negative [16].

Conclusion

Metaplastic breast cancer is a relatively rare disease. To our knowledge this is the first report of p16 positivity in breast cancer in the metaplastic setting. Further prospective multi centre studies are needed to confirm this high level of p16 positivity. Also any relationship between p16 in this setting and HPV needs further elucidation. Therapy of metaplastic breast cancer may also be affected if this proves to be so as p16 by immunohistochemistry is easy to perform.

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