

Immunohistochemical expression of HER2 and MUC1 in breast carcinoma and its correlation with histological grades

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ABSTRACT

Background: Mucin 1 (MUC1) is overexpressed in almost 90% of CA breast cases and is usually associated with poor prognosis. On the other hand, HER2 is profoundly expressed in breast carcinoma and is also linked with increased disease recurrence, tumor invasion and poor prognosis. Thus, this study was designed to assess the expression of MUC1 and HER2 against the histological grades of breast carcinoma in our population.

Materials and methods: It was a cross sectional analytical study conducted at Post Graduate Medical Institute/General Surgery Departments of Lahore General Hospital (LGH), Lahore. Duration of study was 12 month from 27-3-2018 to 30-3-2019. Total 60 newly diagnosed cases of CA breast were included in the study. Immunohistochemistry technique was used and MUC1 and HER2 markers were applied to check the probable association between the two markers and the histological grades of cancer.

Results: A total of 60 cases were included in the study, out of which 96.7% (n=58) were invasive ductal carcinoma, while 3.3% (n=2) were Invasive lobular carcinoma. Among the total, 60% (n=36) were found positive for MUC1 (moderate/strong) and 40% (n=24) were found negative (negative/weak). Regarding HER2, 26.7% (n=16) were strong positive, 20% (n=12) were at the borderline/equivocal and 53.3% (n=32) were found negative for HER 2.

Conclusion: Statistically significant association was found between MUC1 and HER2 against the histological grades of breast cancer patients (p <0.05).

Keywords:

Carcinoma breast, MUC1, HER2, Immunomarker

INTRODUCTION

Breast carcinoma (CA breast) is the most common cancer among females world-wide and second leading death cause after lung carcinoma¹⁻⁴. It is a heterogeneous malignancy with diverse morphologies and clinical implications^{3,5-8}. It is the leading cause of death among females in most developed, under developed and developing countries^{5,8}.

Among Pakistani females, the most frequently diagnosed cancer is the CA breast. Pakistan, as compared to other Asian countries, has the highest number of populations suffering from it. Risk factors that play a major role in Pakistan are lack of awareness, poor socio-economic status, advance age, dietary factors, usage of oral contraceptives and family history. Pakistan is a country where a large bulk of population lives below the poverty line. People in the rural areas are less caring and concerned about their health since they usually do not have ample money to spend on their health. At the same time, it is not easy to make them

aware of any disease, specially breast cancer, due to limitations that the females observe in rural areas¹.

MATERIALS AND METHODS

It was a cross sectional analytical study done at the Department of Pathology Post Graduate Medical Institute (PGMI), Lahore. Specimens were collected from PGMI Affiliated hospital, General Surgery Departments of Lahore General Hospital (LGH), Lahore in a span of 12 months. The sample size of 60 cases was estimated by using 95% confidence level and 90% power. The expected percentage of positive cases detected by HER2 and MUC1 are 46.7 and 75% respectively^{9,10}.

Female patients of ages 30-60 with primary diagnosed breast carcinoma were included in the study, while patients on chemotherapy/radiotherapy/hormone therapy were excluded.

Patients' clinical history was recorded. The biopsy specimens were collected in properly labeled jars containing 10% neutral buffered formalin solution. Detailed gross examination of the specimens was done. The type of biopsy, site, laterality, size, cut surface, lymph node status, presence/absence of necrosis and skin changes were recorded on Lab Proforma.

Conflict of Interest: The authors declared no conflict of interest exists.

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The representative sections were taken and were fixed for 24 hours, after which they were processed in an automated processor according to the recommended protocol. After processing, paraffin embedded tissue blocks were prepared. Three to four micron (3-4mm) thick tissue sections were obtained by using rotary microtome¹¹. H&E staining was done on sections¹².

MUC1 immunostaining was done using Mouse Monoclonal ELISA, IHC optimal dilutions was determined. Purified. IgG. Unconjugated¹³. HER 2 staining was done by the method of, Dilution: Polyclonal Rabbit Anti-Human c-erbB-2 Oncoprotein, Code A0485, may be used at a dilution range of 1:600–1:800 or 1:1000–1:1200 when applied on pretreated, formalin-fixed, paraffin-embedded sections of human mammary carcinoma overexpressing the c-erbB-2 oncoprotein using a 20 minutes incubation at room temperature¹⁴.

RESULTS

In this study 60 samples of CA breast were collected using biopsies from mastectomy specimen. IHC was done to determine histological grades of all the samples¹⁴. MUC1 and HER2 staining were tested against histological grades to make correlation. Our study revealed that there was no correlation between histological grades and MUC1 alone (p=0.396) as shown Figure 1. Contrary to that when compared the histological grades against HER2 (Figure 2), a positive correlation was observed (p=0.015).

In this study, frequency distribution according to age 30-45 years was 45% (n=27), 46-60 years was 41.7% (n=25), age 60 years and above were found to be 13.3% (n=8). The frequency distribution according to quadrant was for upper/inner 6.7% (n=4), for lower/inner 3.3% (n=2), for upper/outer 86.7% (n=52) and for lower/outer 3.3% (n=2). Frequency distribution according to laterality was equal on both sides right and left i.e., 50% (n=30). Frequency distribution according to size was, for 1-2cm 16.7% (n=10), for 3-5cm 46.7% (n=28) and for >5cm 36.7% (n=22). Skin changes were present in 6.7% (n=4), while they were absent in 93.3% (n=56). For histological types, invasive ductal carcinoma frequency was 96.7% (n=58), while invasive lobular carcinoma frequency was 3.3% (n=2). Frequencies according to histological grades were 13.3% (n=8) for grade 1, 56.7% (n=34) for grade 2 and 30% (n=18) for grade 3. Frequency distribution according to MUC1 IHC was, negative stain was found in 23.3% (n=14), weak stain was found in 16.7% (n=10), moderate stain

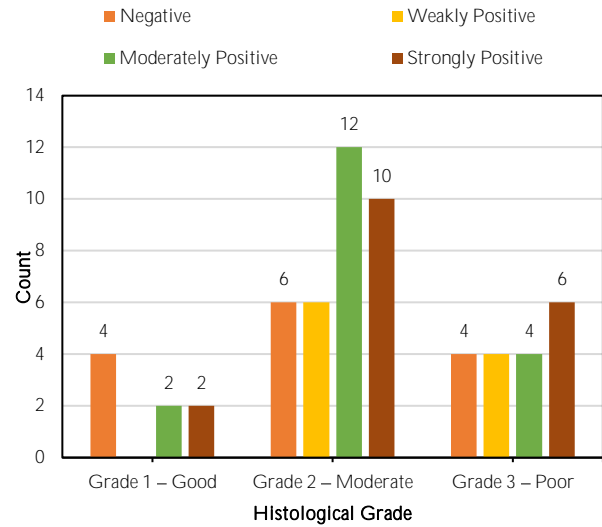


Figure 1: Graphical presentation of comparison regarding Histological Grade & MUC1 IHC. Likelihood Ratio: 6.252. There was no association between histological grade and MUC1 IHC (p = 0.396). Positive predictive value was 60%.

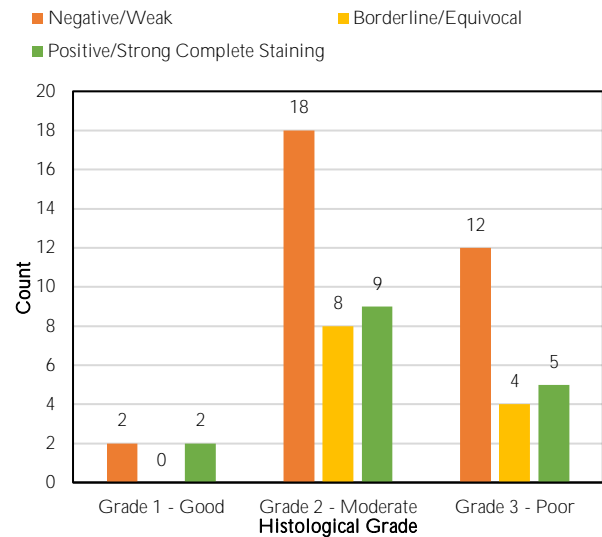


Figure 2: Graphical presentation of comparison regarding Histological Grade and HER2 IHC. Likelihood Ratio: 12.407. There is association between histological grade and HER2 IHC (p = 0.015). Positive predictive value of test was 46.80%

was found in 30.0% (n=18) and strong stain was found in 30.0% (n=18). Frequency distribution according to HER2 IHC was, negative/weak were 53.3% (n=32), borderline/equivocal 20.0% (n=12) and positive/strong-complete staining 26.7% (n=16). Lymph node metastasis was found positive in M30.0% (n=18) and was found negative in 70.0% (n=42) (Table 1).

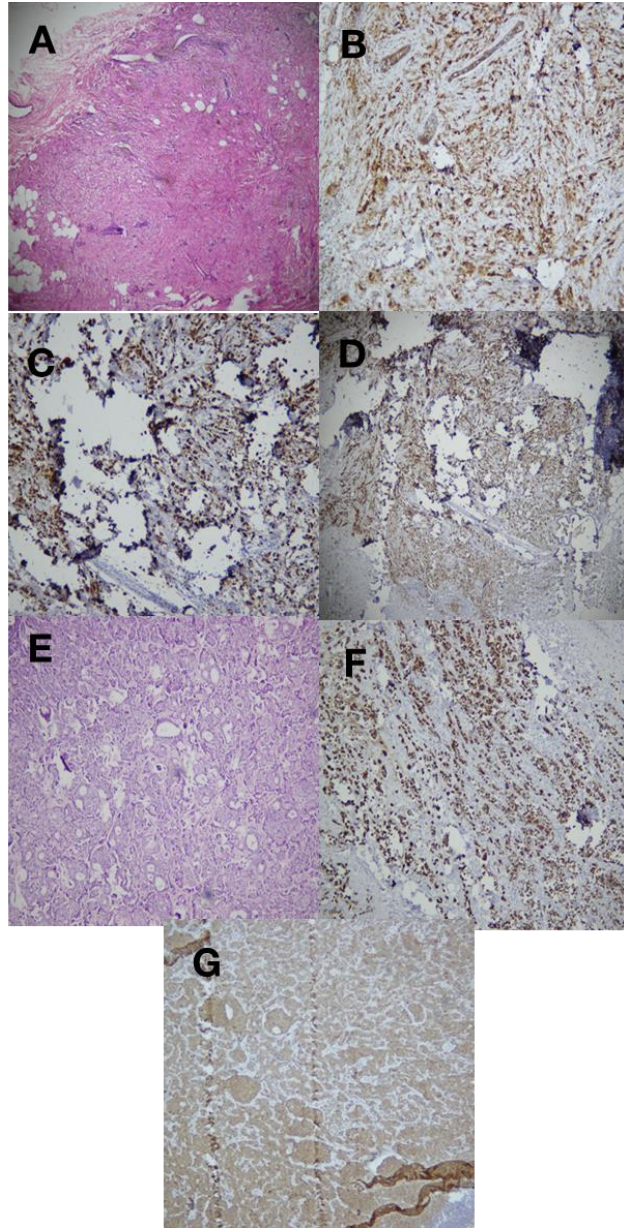


Figure 3: Histopathology

DISCUSSION

CA breast is the malignancy of the breast tissue¹ and can be defined as uncontrolled growth of malignant breast cells that gain access to invade the surrounding tissues and can metastasize to other parts and organs of the body. It presents as a lump in the breast which may be painless, although pain, tenderness and skin changes can also be observed¹⁵.

Exact cause of CA breast is still unknown, it is however thought that few things may be considered as the promoting factors which include age, genetics, weight, positive family history, physical inactivity,

Table 1: Clinicopathological characteristics of cases

Characteristics of 60 Cases	Frequency (%)
Quadrant	
Inner	6 (10.0)
Outer	54 (90.0)
Laterality	
Right	30 (50.0)
Left	30 (50.0)
Encapsulation	
Yes	18 (30.0)
No	42 (70.0)
Size	
1-2cm	10 (16.7)
3-4cm	28 (46.7)
>5cm	22 (36.7)
Cut Surface	
Smooth	18 (30.0)
Rough	36 (60.0)
Irregular	6 (10.0)
Skin Changes	
Yes	4 (6.7)
No	56 (93.3)
Lymph Nodes	
Present	48 (80.0)
Absent	12 (20.0)
Lymph Nodes Number	
No lymph nodes	8 (13.3)
1-2	10 (16.47)
3-4	20 (33.3)
>5	22 (36.7)
Characteristics of 60 Cases	Frequency (%)
Histological Type	
Invasive Ductal Carcinoma	58 (96.7)
Invasive Lobar Carcinoma	2 (3.3)
Histological Grade	
Grade 1 (Good)	8 (13.3)
Grade 2 (Moderate)	34 (56.7)
Grade 3 (Poor)	18 (30.0)
MUC1-IHC	
No Staining	14 (23.3)
Weak Staining	10 (16.7)
Moderate Staining	18 (30.0)
Strong Staining	18 (30.0)
HER2-IHC	
Negative/Weak Staining	32 (53.3)
Borderline/Equivocal	12 (20.0)
Positive/Strong Complete Staining	16 (26.7)
Lymph Node Mets	
Yes	18 (30.0)
No	42 (70.0)
Lymphocytic Infiltrate	
Yes	4 (6.7)
No	56 (93.3)
Age	
30-45	27 (45.0)
46-60	25 (41.7)
61-80	8 (13.3)
>80	None

alcohol consumption, diet and lack of awareness about the disease^{1,2,15,16}, along with some other pathological factors like angiogenesis, metastasis and inflammation which are mediated by the cytokines¹⁷. Moreover, oral contraceptives usage, hormones replacement therapies and prolonged exposure to ovarian hormones can play a pivotal role in CA breast development^{1,18}. Similarly, it is

also observed that increased risk of CA breast development is common in those females who experience early menarche, late age at menopause, late age at the first full term pregnancy, older age at first live births and no breastfeeding. However, for the postmenopausal women an increasing risk with earlier age at menarche was observed. A strong association of increasing breast cancer risk with increasing age at menopause was reported in some studies. High body mass index (BMI) has also been shown to be associated with an increased risk of breast carcinoma in most of the studies ¹.

According to some statistics provided by DeSantis in 2017, it was estimated that in 2017 the probable number of females suffering from CA breast in the US will be 316,120, while 40,610 women were expected to die of this disease the same year. Moreover, it is stated that 81% of CA breast cases are diagnosed in women of age more than 50 years and 89% of deaths occur in the same group due to CA breast ¹⁹. The age-specific pattern is typical of that in developing countries, where the risk of cancer development increases up in the 50s i.e., around menopause ²⁰. GLOBOCAN statistics of 2012 show that in the US almost 1,300,000 women were diagnosed suffering from CA breast, with 522,000 deaths. This also exhibited that since 2008 an increase of almost 18% CA breast cases has been observed. It is also estimated that by 2050 almost 3.2 million new cases of CA breast would be diagnosed annually. This is a worrisome figure that requires immediate and prompt actions to prevent women from this disease ⁷.

Asia alone accounts for a total of 39% of newly diagnosed cases of CA breast in the world with 44% deaths. In our neighboring country India, approximately 25% of all cancers among females is CA breast, with an incidence rate of 25.8/100,000 women and mortality rate of 12.7/100,000 women. Almost 48% CA breast cases in India are below 50 years of age, while increased incidence of CA breast is being observed in women between 30 and 40 years of age ²¹.

On the other hand, in Pakistan 34,038 new cases of CA breast were reported in 2012. A total of 16,232 deaths occurred the same year due to CA breast ²². Similarly, Pakistan also accounted for the highest standardized death rate in whole of Asia the same year, which was 25.5/100,000 ^{1,22,23}. Almost 1 in every 9 women is suffering from CA breast in Pakistan, making it the most common malignancy among Pakistani females ^{1,23}.

According to a WHO press release, the CA breast accounts for 24% of all cancer cases, where 1 in every 4

new cancer cases in women is of breast cancer ²⁴. According to data shared by WHO in 2011, more than 1.3 million cases were diagnosed and almost 0.5 million deaths occurred globally, the same year ²⁵.

CONCLUSION

Early diagnosis with the help of HER2 and MUC1 may help to reduced mortality and morbidity of CA breast population targeted therapy should be used.

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