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Immunohistochemical Expression of Phosphatase and Tensin Homolog In Relation To Molecular Classification of Carcinoma Breast

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Abstract

Background: Breast carcinoma is the most common and deadly malignancy of women globally. Tumor suppressor genes play a significant role in maintaining genome integrity and the cell cycle. Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene which is a negative regulator of PI3K/Akt signalling pathway and has a major role in breast carcinogenesis.

Objective: The purpose of this study was to assess immunohistochemical expression of phosphatase and tensin homolog in relation to molecular classification of carcinoma breast.

Methodology: This descriptive study was conducted on 100 modified radical mastectomy specimens received in the department of pathology, PGIMS, Rohtak over a period of one year. Immunohistochemical expression of PTEN gene, ER, PR and Her2neu was determined using specific monoclonal antibodies. PTEN staining was assessed by examining the nuclear and cytoplasmic staining of tumor cells with normal breast epithelial tissue taken as positive control. PTEN expression status was correlated with various clinicopathological parameters including age, tumor size, tumor type, lymph node status, histologic grade and Nottingham Prognostic Index (NPI).

Results: The present study included 100 invasive carcinoma breast cases, out of which 41 (41.0%) were triple negative, 15 (15.0%) Luminal A, 12 (12.0%) Luminal B, 17 (17.0%) Her2 neu enriched type and 15 (15.0%) cases could not classified into any of the

molecular subtype of breast cancer. 52/100 (52.0%) of cases had decreased expression while its expression retained in 48/100 (48.0%) of cases. Loss of expression was significantly associated with histological grade of tumor and Nottingham Prognostic Index (NPI). However, loss of PTEN expression did not correlate with age, side and size of tumor and lymph node metastasis.

Conclusion: Loss of PTEN expression can be assessed using immunohistochemistry. PTEN loss can predict worse prognosis and poor survival in breast cancer patients.

Keywords: Phosphatase and tensin homolog, breast carcinoma, immunohistochemistry, NPI, prognosis, survival.

Introduction

Breast carcinoma is the most common and deadly malignancy of women globally. Risk factors include female gender, increasing age, strong family history, germline mutation, early menarche (<12years), late menopause (>55years), high alcohol consumption.[1] In developing countries, majority of the patients come at an advanced stage, where the patient management options are neoadjuvant chemotherapy before definitive surgical therapy.[2] To improve early diagnosis and prognosis as well as to provide the most effective drug suitable with molecular characteristics of the patients, identification of biomarkers and gene expressions is needed.[3]

PTEN (Phosphatase and Tensin Homolog) is a novel tumor suppressor gene and is located on the chromosome 10q23.1.[4] PTEN is a negative regulator of PI3K/AKT signalling pathway. It dephosphorylates the 3' end of the triphosphate PIP3 in the inositol ring, resulting in the biphosphate PIP2, which inhibits AKT activation and downstream signalling processes that depend on AKT for activation. Inactivation of PTEN has been associated with tumorigenesis in multiple human cancers, including breast cancer.[5]

In our study, we attempted to assess immunohistochemical expression of PTEN in relation to molecular carcinoma of breast. This study will be helpful in improving our understanding in role of biomarker PTEN in the biology of breast carcinogenesis.

Material and method

Case selection: The present descriptive study was conducted on 100 modified radical mastectomy specimens received in the Department of Pathology, PGIMS, Rohtak during a period of one year. Exclusion criteria included trucut biopsy, lumpectomy, cases with incomplete information and inadequate biopsies.

Morphological evaluation

The tissue was fixed in 10% formalin, processed for histopathological examination and representative sections were stained with Haematoxylin and eosin (H&E). Tumor size, lymph node status, Nottingham modification of Bloom-Richardson grading (histologic tumor grading) and Nottingham Prognostic Index (NPI) was noted in every case.

Immunohistochemical analysis (IHC)

Representative section from each case were subjected to immunohistochemical staining for PTEN, ER/PR and Her2neu. Positive and negative control were run simultaneously.

PTEN staining interpretation.

Cytoplasmic and nuclear expression was graded according to the percentage of positive tumor cells:

-: 0%

+: <20%

++: 20-50%

+++: >50%

Grades – or + were considered as low-level expression and ++ or +++ were considered high level expression.

Control:

The adjacent normal breast epithelial tissue was used as positive control and negative control was obtained by substituting the primary antibody with the antibody of non-specific relevance.

IHC expression of ER/PR was assessed with Allred scoring system described in most recent American Society of Clinical Oncology/College of American Pathologists guidelines.

Her2neu staining:

Intense membranous staining of >10% of the tumor cells was taken as positive.

The PTEN expression was correlated with clinicopathological parameters like age of the patient, tumor size, tumor grade, tumor type and axillary lymph node status.

Statistical Analysis

A descriptive study was carried out for 100 cases of breast carcinoma. The collected data was analysed with the help of software package (SPSS version 20.0). Statistical comparisons were made using Chi-square test. P value <0.05 was taken as significant.

Results

Distribution of cases according to clinicopathological parameters:

A total of 100 breast cancer cases were taken out of which 41 (41.0%) were triple negative, 15 (15.0%) Luminal A, 12 (12.0%) Luminal B, 17 (17.0%) Her2 neu enriched type and 15 (15.0%) cases could not be classified into any of the molecular subtype of breast cancer. The average age of patients was 52.1±2.6; and minimum and maximum ages were 28 and 88 years respectively. (Fig.1) 52(52.0%) cases showed right laterality. On the basis of tumor size, 18(18.0%) were ≤2cm, 65(65.0%) were between 2-5cm and 17(17.0%) cases were >5cm. The tumors were histological grades I, II and III in 20, 55 and 25 respectively. Majority of the cases 52 (52.0%) were in the moderate prognosis NPI category. Lymph node metastasis was seen in the 66(66.0%) cases while 34(34.0%) cases were node negative. Lymphovascular invasion was identified 45(45.0%) cases and was absent in 55(55.0%) cases. DCIS was present with invasive tumor in 21(21.0%) cases and 79(79.0%) cases had no DCIS component. 27(27.0%) cases were positive for ER and 30(30.0%) cases were positive for PR. Her2 neu expression was seen in 20(20.0%) cases.

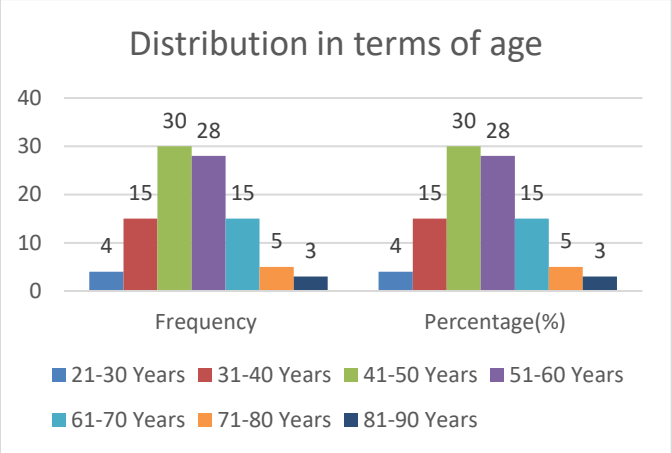

Figure 1: Distribution of the cases in terms of age. (n=100)

PTEN expression by immunohistochemistry:

The PTEN expression was seen in nucleus and cytoplasm of the tumor cells as well as normal duct epithelial cells and myoepithelial cells. Out of 100 invasive breast cancer cases loss of PTEN expression was seen in 52(52.0%) cases. (Fig.2)

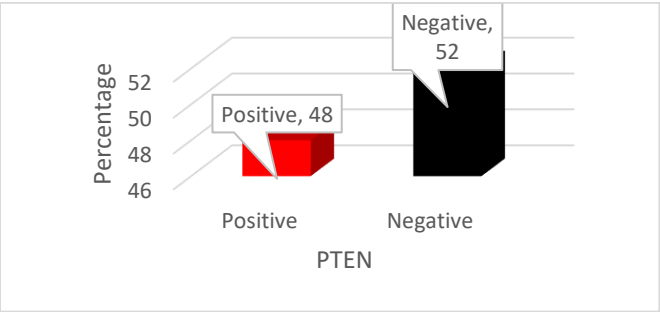

Figure 2: Distribution of the cases in terms of PTEN expression (n=100)

Table 1: Correlation between PTEN expression and Clinico-pathological parameters

Parameters	Frequency (%)	PTEN Positive(n=48)	PTEN Negative (n=52)	p value
Age (years)				
21-30	4	2 (4.2%)	2 (3.8%)	0.352
31-40	15	7 (14.6%)	8 (15.4%)	
41-50	30	12 (25.0%)	18 (34.6%)	
51-60	28	12 (25.0%)	16 (30.8%)	
61-70	15	9 (18.7%)	6 (11.5%)	
71-80	5	4 (8.3%)	1 (1.9%)	
81-90	3	2 (4.2%)	1 (1.9%)	
Side				
Right	52	24 (50.0%)	28 (53.8%)	0.701
Left	48	24 (50.1%)	24 (46.2%)	
Tumor Size				
≤2	18	10 (20.8%)	8 (15.4%)	0.694
2-5	65	31 (64.6%)	34 (65.4%)	
>5	17	7 (14.6%)	10 (19.2%)	
Histological Grade				
I	20	13 (27.1%)	7 (13.5%)	<0.001
II	55	31 (64.6%)	24 (46.1%)	
III	25	4 (8.3%)	21 (40.4%)	
NPI Category				
Good	15	11 (22.9%)	4 (7.7%)	0.005
Moderate	52	25 (52.1%)	27 (51.9%)	
Poor	33	12 (25.0%)	21 (40.4%)	
Lymph Node involvement				
0 nodes	34	19 (39.6%)	15 (28.8%)	0.623
1-3 nodes	27	13 (27.0%)	14 (26.9%)	
4-9 nodes	21	8 (16.6%)	13 (25.1%)	
≥10 nodes	18	8 (16.6%)	10 (19.2%)	
Lympho-vascular invasion (LVI)				
Present	45			
Absent	55			
DCIS				
Present	21			
Absent	79			

Loss of PTEN expression was statistically significant with histologic grade (p-value <0.001) and NPI Category (p-value 0.005).

Table 2: Correlation between PTEN expression and molecular subtype of breast carcinoma:

Molecular subtype	Frequency (%)	PTEN positive (%)	PTEN negative (%)	p-value
Luminal A	15	9 (18.8%)	6(11.5%)	0.537
Luminal B	12	6 (12.5%)	6 (11.5%)	
Her2 enriched	17	6 (12.5%)	11 (21.2%)	
Basal	41	18 (37.5%)	23 (44.2%)	
Unclassified	15	9(18.8%)	6 (11.5%)	

Maximum number of negative PTEN cases (44.2%) were of basal subtype.

11.5% negative PTEN cases could not be classified into any of the molecular subtype.

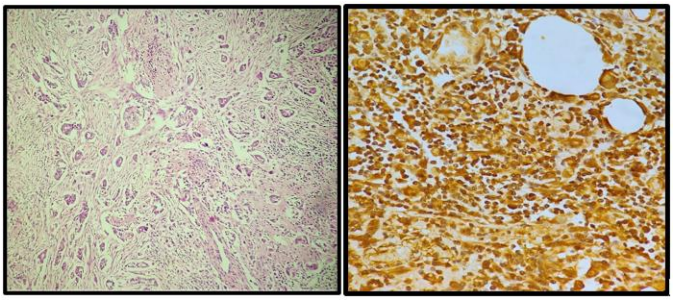

Figure 3: Grade I infiltrating ductal carcinoma showing nuclear and cytoplasmic expression of PTEN.

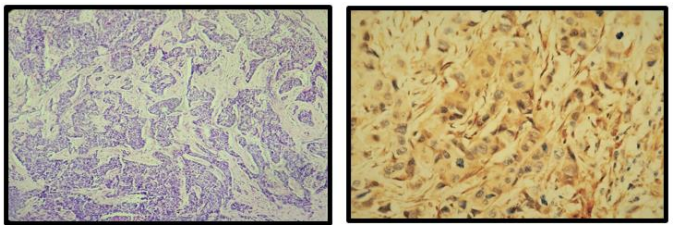

Figure 4: Grade II infiltrating ductal carcinoma showing cytoplasmic expression of PTEN expression.

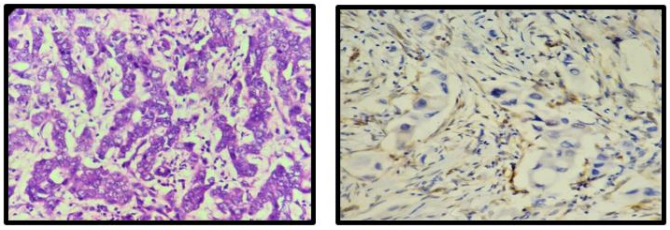

Figure 5: Grade III infiltrating ductal carcinoma showing loss of PTEN expression.

Discussion

Breast cancer is the most common malignancy in women around the world, with an increasing incidence and a high mortality rate. In developing countries, many breast cancer patients come at an advanced stage of the disease, where the patient management options are neoadjuvant chemotherapy before definitive surgical therapy.[2] Identification of biomarkers and gene expressions are needed to improve early diagnosis and prognosis, as well to provide the most effective drug suitable with molecular characteristics of the patients. Studies have indicated that prognostic and predictive biomarkers are molecules involved in the regulation of cellular mechanisms, including proliferation, apoptosis, angiogenesis, metastasis and therapeutic resistance.[3]

PTEN is a tumor suppressor gene that play a role in breast cancer development.[3] PTEN is a negative regulator of PI3K/AKT signalling, directly and indirectly affecting cell survival, proliferation and apoptosis. Inactivation of PTEN has been associated with tumorigenesis in multiple human cancers.[6]

The present study is conducted to understand the importance of PTEN as a tumor suppressor gene during breast cancer growth, invasion and metastasis, furthermore, immunohistochemical expression of PTEN and its correlation with clinicopathological parameters in breast cancer as being important in tumorigenesis, local invasion and metastasis.

In our present study, PTEN loss had been detected in 52 (52%) of the cases. This result is in agreement with Gonzalez-Angulo[7] et al who found 55% PTEN loss in breast cancer cases using immunohistochemical methods. Dean et al[8] found loss in 48.3% cases, Depowski et al[9] observed loss in 48% breast cancer cases. The results of various studies demonstrated reduced PTEN expression ranging from 33.0% to 55.0% by immunohistochemical methods.

Loss of PTEN had been occurred more frequent in the age group of 41-50 years. No significant association was seen between PTEN loss and age. Our results were in concordance with the studies by Zhang et al[10] and Chang et al[11] In our study, 20% cases were in histological Grade I, 55% cases in Grade II and 25% cases in Grade III. Majority of PTEN negative cases (46.1%) were under Grade II. Statistically significant association was seen in PTEN loss and tumor histologic grade (p value <0.001). Our results are in concordance with results of Chang et al[11] and Golmohammadi et al[12] having maximum number of cases with histologic Grade II. While studies by Altemimi et al[13] had maximum number of cases in histologic Grade III. It is probable that due to the removal of inhibitory factors from the cycle of cell division in malignant cells without PTEN gene expression, the rate of division and invasiveness in them increases significantly.

It was observed that PTEN loss was maximum (28.8%) in the cases with no lymph node metastasis of breast cancer, cases with 1-3 lymph node involvement showed 26.9% loss. Lowest PTEN loss (19.2%) was seen in the cases with ≥10 lymph nodes involvement. There was no significant association was seen between PTEN loss and lymph node involvement. Our results are similar with the results of Zhang et al.[10] However, contrary to our

study, studies by Altemimi et al[13], Shoman et al[14], Chang et al[11] and Depowski et al[9] had significant association with PTEN loss and lymph node metastasis. No plausible explanation could be found to explain this discordance in our study.

A significant association was found between PTEN loss and NPI category (p value-0.005) with maximum number of cases (51.9%) negative for PTEN were in the moderate prognosis group. These findings agree with the reported by Altemimi et al[13], Windarti et al[3] and Golmohammadi et al[12] pointing that besides the investigation of new markers, pathological parameters which are usually used in the prognosis are important.

In this study, we have maximum number of cases showing PTEN loss (44.2%) in the basal molecular subtype of breast cancer. Our findings are similar with that of Altemimi et al[13] and Li et al[4] where basal subtype i.e triple negative breast cancer cases showed maximum loss of PTEN. It indicated that PTEN loss might contribute to the progression of breast cancer and is associated with poor prognosis.

In conclusion, the present study showed that loss of PTEN can be readily assessed using immunohistochemistry. Continuous lines of research that focuses on different forms of carcinogenesis underlining the growth of breast carcinoma has been established. Comprehensive analysis of the PTEN gene has indicated that not only it is a tumor suppressive gene, but also it plays a role in breast carcinoma prognosis. The combined immunohistochemical analysis of PTEN, ER, PR and HER2 NEU may provide additional data to perform a tailored risk assessment while evaluating breast cancer patients.

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