Relationship between Phosphatase and Tensin Gene Expression and Clinicopathologic Features of Breast Cancer in Patients who Underwent Biopsy or Breast Surgery

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Received: 05 Jan. 2017; Received in revised form: 18 Feb. 2017; Accepted: 09 Mar. 2017

Abstract

Background: Phosphatase and tensin (PTEN) gene is a tumor suppressor gene on chromosome 10q23 that is composed of 11 exons. Several studies have shown that loss of PTEN function is a common occurrence in breast cancer in particular in triple negative type, and it is significantly associated with age and higher stage of cancer. In this study, the expression of this gene in malignant breast cancer tissue samples and their correlation with clinicopathologic parameters was studied.

Methods: In this retrospective study, 65 malignant tissue samples were chosen for immunohistochemistry (IHC) test. Other information about clinicopathologic features were collected from pathology reports and patients' medical records. IHC on the selected paraffin blocks was performed, and the collected data were analyzed using SPSS software and chi-square test. $P < 0.0500$ was considered statistically significant.

Results: PTEN expression rate in malignant breast tissue was 50.8% of the cases (33 out of 65 samples). Lack of PTEN expression had significant correlation with involvement of the lymph node sent by the sample, vascular or perineural invasion, metastasis and chemotherapy background, spontaneous malignancy presence, familial history, negative progesterone receptor, negative estrogen receptor, and positive her2/neu. No relationship was observed between the expression of PTEN with patients' age, tumor size, age group of the patients after categorization into two groups of under 50 years and over 50 years, lesion location (left or right breast), and tumor grade.

Conclusions: The results showed PTEN loss as a frequent event in breast cancer that is closely associated with progression and poor prognosis. PTEN loss might predict more aggressive behavior and worse outcomes in patients with breast cancer.

Citation: Mohammadi-Vajari MA, Samizadeh E, Sadeghian Y, Mohammadi-Vajari E, Sadeghian E, Lashkari MH. **Relationship between Phosphatase and Tensin Gene Expression and Clinicopathologic Features of Breast Cancer in Patients who Underwent Biopsy or Breast Surgery.** *Acad J Surg*, 2017; 4(1): 16-9.

Keywords: Phosphatase and tensin protein; Gene expression; Pathological fracture; Breast cancer

Introduction

Phosphatase and tensin (PTEN) gene is a tumor suppressor gene on chromosome 10q23 that is composed of 11 exons (1). PTEN homolog on chromosome 10 is one of the most common tumor suppressors that are mutated in human cancers. PTEN catalyzes converting phosphatidylinositol (3,4,5)-trisphosphate into phosphatidylinositol (4,5)-bisphosphate lipid membrane and is the main mediator of AKT/protein kinase B pathway. Inherited PTEN mutations increase susceptibility to Cowden syndrome (syndrome with autosomal dominant inheritance pattern characterized by hamartomas, and increased susceptibility to breast cancer and thyroid cancer), which is associated with 25-50 percent increase in risk of susceptibility to breast cancer.

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Furthermore, PTEN somatic mutations are associated with different types of cancers in which glioblastoma is considered as the most common type (2-4).

Several studies have shown that loss of PTEN function is a common occurrence in breast cancer in particular in triple negative type, and it is significantly associated with age and higher stage of cancer (5,6). Furthermore, several studies have found that patients with PTEN gene expression are sensitive to the doxorubicin, while patients without or with reduced expression of this gene are resistant to the doxorubicin (7). Other studies stated that loss of PTEN function may be associated with resistance to the herceptin (trastuzumab). Herceptin (trastuzumab) can lead to improved prognosis in positive her2 cancer patients; however, some patients do not respond well to this drug or are resistant to it; it is reported that lack of PTEN may be responsible for the poor response (8,9). Loss of PTEN function has been reported in 15-65% of positive her2 breast cancers (10).

The role of this gene in breast cancer when it is next to genes such as BRCA1 and P53 is neglected. Recent studies have considered the importance of this gene in early diagnosis and treatment of breast cancer, especially triple negative types (11). Given the importance and prevalence of breast cancer in different communities as well as by the importance that the expression or non-expression of this gene can be used in treatment and prognosis of patients with breast cancer, in this study, the expression of this gene in malignant breast cancer tissue samples and their correlation with clinicopathologic parameters was studied.

Materials and Methods

This study is a retrospective, descriptive study on patients who underwent biopsy or breast surgery during 2012-2015 (since the early 2012 to the late 2015) in Imam Reza Hospital (501 Artesh of Islamic Republic of Iran). To do this, referring to the hospital pathology ward and examining samples' registries, all pathological reports of the patients in this period available in hospital pathology ward were studied, and samples prepared from breast tissue were separated. Then, data collection form was completed based on the information in pathology reports, including patients' age, sex, tumor location, sample size, final diagnosis and tumor size, tumor grade, Nottingham score, lymph node involvement, and vascular or perineural invasion in the case of tumoral tissues. Meanwhile, a number of samples were deficient in information that was corrected and completed by referring to patients' admission records at the archives and phone call to patients. Finally, 188 patients were included in the study with 230 samples. Among the samples, 65 malignant tissue samples were chosen for immunohistochemistry (IHC) test.

IHC on the selected paraffin blocks was performed with the help of Professor Kamalian's Laboratory. The specimens were fixed in formalin (10%). Tissue passage was performed by tissue processing device, and after infiltration of the samples in melted paraffin, the samples were embedded in paraffin. 4 µ were prepared from all samples with litze microtome. The slides containing the samples were stained with hematoxylin and eosin; the slides were reviewed by two expert pathologists (double-blind). PTEN gene expression in samples was performed as follows. Temperature and concentrations of antibodies for IHC was performed according to the kit protocol, but for mask removal of the antigenic characteristics of the samples' areas, the microwave and citrate buffer was used at 100 °C for 10 minutes. To inhibit the endogenous peroxidase activity, it was placed in 3% hydrogen peroxide for 30 minutes and then the slides were 5 times washed by phosphate saline buffer. The primary antibody (primary specific rabbit monoclonal PTEN antibody) Novocastra (Newcastle, UK) was dropped on the slides and were washed 3 times with phosphate saline buffer and then the secondary antibody was used. The slides were washed 3 times by phosphate saline buffer to cross to the next stage. Streptavidin connected to horseradish peroxidase which is capable of emitting diaminobenzidine was used to stain the cells, and it was analyzed by an optical microscope and the image was taken. The cells stained < 10% were considered negative, those with more than 10% of brown color were considered positive, and taking into account the expression of the PTEN, the slides were divided into positive and negative degrees. The collected data were analyzed using SPSS software (version 23, IBM Corporation, Armonk, NY, USA) and chi-square test, and $P < 0.0500$ was considered statistically significant.

Results

A total of 188 patients (mean age 51.5 years) were included into the study with 230 samples. Among the samples, 65 malignant tissue samples were chosen for IHC test.

PTEN expression rate in malignant breast tissue was 50.8% of the cases (33 out of 65 samples). As shown in table 1, in our study, lack of PTEN expression had a significant correlation with involvement of the lymph node sent by the sample, vascular or perineural invasion, metastasis and chemotherapy background, spontaneous malignancy presence, familial history, negative progesterone receptor, negative estrogen receptor, and positive her2/neu. In our study, no relationship was observed between the expression of PTEN with patients' age, tumor size, age group of the patients after categorization into two groups of under 50 years and over 50 years, lesion location (left or right breast), and tumor grade (Table 2).

Table 1. Relation between PTEN expression and clinicopathologic features

Variables	Levels	Total	PTEN negative	PTEN positive	P-value
		Count (%)	Count (%)	Count (%)	
Age group	< 50	32 (100)	16 (50)	16 (50)	1.0000
	> 50	33 (100)	16 (48.5)	17 (51.5)	
Grade	1	6 (100)	4 (66.7)	2 (33.3)	0.5480
	2	32 (100)	14 (43.8)	18 (56.3)	
	3	27 (100)	14 (51.9)	13 (48.1)	
Lymph node	Positive	35 (100)	32 (91.4)	3 (8.6)	< 0.0010
	Negative	30 (100)	0 (0)	30 (100)	
Vascular or perineural	Positive	36 (100)	32 (88.9)	4 (11.1)	0.0510
	Negative	2 (100)	0 (0)	29 (100)	
Metastasis	Positive	36 (100)	32 (88.9)	4 (11.1)	0.0210
	Negative	29 (100)	0 (0)	2 (100)	
ER, PR	Positive	38 (100)	۷7 (18.4)	31 (81.6)	< 0.0010
	Negative	27 (100)	25 (92.6)	2 (7.4)	
Her2/neu	Positive	35 (100)	29 (82.9)	6 (17.1)	0.0040
	Negative	30 (100)	3 (10)	27 (90)	
Synchronous malignancy	Positive	35 (100)	29 (82.9)	6 (17.1)	0.0450
	Negative	30 (100)	3 (10)	27 (90)	
Family history	Positive	35 (100)	31 (88.6)	4 (11.4)	0.0340
	Negative	30 (100)	1 (3.3)	29 (96.7)	
Chemotherapy	Positive	32 (100)	29 (90.6)	3 (9.4)	0.0012
	Negative	33 (100)	3 (9.1)	30 (90.9)	
Location	Left side	29 (100)	16 (55.2)	13 (44.8)	0.4590
	Right side	36 (100)	16 (44.4)	20 (55.6)	

PTEN: Phosphatase and tensin

Discussion

PTEN expression in different studies has been reported from 48% in Sara's study to 70% in Golmohammadi et al.'s study (12). Gonzalez-Angulo et al. (13) examined the role of PTEN in the metastasis of breast cancer. In this study, as well as the current study, IHC technique was used to study gene expression and the results indicated that there was a significant difference between PTEN gene expression in metastatic and non-metastatic samples. In other words, the lack of PTEN expression was associated with a higher metastasis that this study as well as other mentioned studies is consistent with the results of our study. PTEN gene plays a role in the development of synchronous malignancies; Cowden syndrome can be regarded as a good example in this field, as well as in several studies, synchronization of ovarian and endometrial cancer have been investigated in patients with mutations in this gene. For example, Lin et al. (14) have been studied the various mutations of PTEN and its relation to simultaneous endometrial and ovarian cancer. In our study, there was a statistically significant correlation between PTEN expression and simultaneous malignancy in patients with breast cancer. Of course, this needs to be confirmed by prospective and more accurate studies to distinguish between metastasis and simultaneous malignancy, accurately. In Lee-Hoeflich et al. study (15), the expression of PTEN and her2 was investigated that its results as of the results of the current study indicated that PTEN status in breast cancer is differed significantly between her2-positive and her2-negative groups. In Yang et al.'s study (16), the loss of PTEN function in malignant cases was more than benign tumors. In addition, there was an inverse relationship between PTEN expression and tumor stage, cell malignancy stage, axillary lymph node involvement, recurrence, and metastasis. These results are consistent with the results of the current study. Limitations such as small malignant sample size, defective recording of medical documents, and limitation in preparing the experiment materials existed in our study.

Table 2. Relation between PTEN with age and size

Variables	Index	PTEN positive	PTEN negative
Size	Mean ± Standard deviation	3.385 ± 3.530	3.219 ± 2.420
	Count	33	32
	P value	0.826	
Age	Mean ± Standard deviation	51.515 ± 13.650	51.656 ± 13.460
	Count	33	32
	P value	0.967	

PTEN: Phosphatase and tensin

Disaffiliation between PTEN and patients' age, tumor size, age group of the patients after categorization into two groups of under 50 years and over 50 years, lesion location (left or right breast), and tumor grade could be due to the following reasons: (1) difference in sample size, (2) difference in agreed percent for confirming positivity of these markers expression, (3) difference in technique and method of performing IHC, and (4) applying different classifications for clinicopathologic index.

Although the current study is subject to some limitations, its results identify PTEN loss as a frequent event in breast cancer that is closely associated with progression and poor prognosis. PTEN may be a promising, useful biomarker for predicting clinical outcomes in women with breast cancer.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

Hereby, I would like to thank and appreciate the Vice President of Research and President of the Army University of Medical Sciences, President and Staff of the Department of Pathology in Imam Reza Hospital of Army of Islamic Republic of Iran, and President and Staff of the Professor Kamalian's Laboratory for cooperation in the stages of the project.

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