



Low expression of PTEN gene is an important trigger event for progression of breast carcinoma

***Hussein. A. Al-hamadawi** □ College of Education /University of Al-Qadisiya

****As'ad. A. Al-Janabi** College of Medicine / University of Kufa

**** Thekra A Al-Kashwan** College of Medicine / University of Kufa

***Adnan.w. Al-bider1.** College of Medicine / University of Al-Qadisiya

Abstract

PTEN tumor suppressor gene is located on chromosome 10q23, consisting of 9 exons, that encodes a 403 amino acids dual-specificity phosphatase with lipids and protein phosphatase activities. PTEN plays a major role in control multiple cellular functions such as cell metabolism, cell cycle progression and cell survival. The PTEN gene is frequently mutated or inactivated in high proportion of human cancer including breast cancer. The aim of this study was to find the correlation between the decrease of PTEN expression with clinicopathological parameters in breast cancer patients.

The current study included 85 sample of Paraffin-embedded breast cancer tissues were analyzed for PTEN expression by immunohistochemistry. We also demonstrated correlation PTEN expression and clinicopathological factors.

The loss expression of PTEN protein was found in 50 (58.8%) of 85 breast cancer tumors, while PTEN protein expression was normal in 35 (41.2%) of 85 tumors. The loss expression of PTEN was correlation with lymph node involvement and age patient, and there was no significant correlation with stage, grade, tumor sizes and histological types.

Conclusion : These results demonstrated the loss of PTEN expression was found in more than half of breast cancer cases and the reduced expression of PTEN protein was correlated with lymph node metastases and may play a role in progression of breast carcinoma , and hence , worse prognosis in the patients with breast cancer. Beside, the detection of the loss of PTEN expression may serve as a useful biologic marker for progression in invasive breast cancer.

Introduction

PTEN tumor suppressor gene is located on chromosome 10q23, consisting of 9 exons, that encodes a 403 amino acids dual-specificity phosphatase with lipids and protein phosphatase activities. PTEN has been demonstrated to play a significant role not only in cell cycle regulation and apoptosis, but also in cell adhesion, migration and differentiation ,However, PTEN also plays a major role in control multiple cellular functions such as cell metabolism (1,2 and 3).PTEN is a regulator of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B(Akt) signaling pathway by converting phosphatidylinositol-3,4,5-triphosphate(secondary messenger) to phosphatidylinositol-4,5-biphosphate, thereby acting in opposition to PI3K pathway that is involved in regulating growth, proliferation and survival(4,5 and 6).

Genetic alterations at PTEN locus has a been described in a variety of neoplasms, including primary central nervous system, breast , prostate, colon and bladder tumors (7,8,9,10,11 and 12). The PTEN gene is frequently mutated or inactivation in proportion up to 30% of breast cancer. Germline mutations in *PTEN* gene was identified in Cowden syndrome (CS), which is characterized by a high risk for development of breast cancer. In families with CS, 80% have PTEN germline mutations and female CS patients have a 25-50% lifetime risk of developing breast cancer(13 and 14).in addition the incidence of PTEN mutation is relatively high in patients with sporadic breast cancer (15). The recent immunohistochemical studies demonstrated a reduced expression of PTEN protein in 30–50% of breast cancer specimens(16 and 17). Loss of PTEN maybe play an important role in development of sporadic breast carcinoma (16). These results indicate that PTEN is important for the tumorigenesis, development and prognosis of breast cancer. In our current study tried show frequency loss of PTEN expression, it's correlation with clinicopathological factors in Iraqi breast cancer patients.

Material and methods

Patients and tissue samples.

Paraffin-embedded tissues were randomly selected from 85 breast cancer patients over period from 2012-2014. All the cases of breast cancer included in this study were collected from the private laboratories and the laboratories of AL-sadr Teaching hospital in Najaf Al-ashraf Province. Their ages were ranging from 25 to 81 years, with a mean age of (44.5) years. confirmation of histopathological diagnosis, grade and stage of tumor was carried out after reviewing all slides before proceeding to immunohistochemical approach.

Immunohistochemistry Analysis.

Paraffin-embedded sections (5m) of tumor blocks were placed on positively charged slides. These sections were deparaffinized with xylene , rehydrated in serial alcohol solutions and were pre-treated with antigen retrieval solution (0.01 M, citrate buffer, pH9.0, DakoCytomation/Denmark) in water-bath at 95°C for 30 minutes.

The sections were incubated in 0.3% hydrogen peroxide for 10 min to block the endogenous peroxidase activity. The slides were incubated with Monoclonal Mouse Anti-Human PTEN Protein(**0.2ml, Clone 6H2.1, Code M3627**, Dako North America) (dilution 1:50) 30 min in a humidified chamber at 37c. The slides were subsequently incubated with a biotinylated universal secondary antibody and with Streptavidin-Biotin horseradish peroxidase label. After, the sections were incubated with 3,3'-diaminobenzidine (DAB) substrate chromogen solution and counterstained with hematoxylin. sections of breast cancer tissue well known to be positive for **PTEN** were used as positive control for each run of immunostaining while negative control slides were incubated with phosphate buffered saline (PBS) instead of primary antibody. The normal epithelial duct and myoepithelial cells were used as internal control as well as benign tumor tissues were regards as control group for PTEN expression.

Immunostaining scoring

Evaluation of PTEN expression was semiquantitative based on staining intensity and distribution according to previous studies (18 and 19): Distribution was scored as diffuse (>50% tumor staining), regional (15-50% tumor staining) and focal

(<15% tumor staining). Intensity staining was scored was in comparison to the internal positive control was follows : strong (staining equal to or stronger than the internal positive control), moderate (less than the internal positive control but still positive staining) and weak (no or no expression).

Tumors cells consider as positive for PTEN expression include intense reactivity with any distribution and moderate to diffuse staining, whereas tumors showing moderate intensity to regional, moderate to focal, or weak staining with any distribution were considered as negative for PTEN expression. therefore; the scoring represent in the following:

score 0: weak intensity with diffuse, regional and focal distribution.

score1: moderate intensity with regional and focal distribution.

score 2: moderate intensity with diffuse distribution.

score 3: strong stain with diffuse, regional and focal distribution

Statistical Analysis.

The Fisher's exact probability and Odds ratios (ORs) were calculated using the Statistical Package of Social Science program (SPSS for windows, version 20.0) in order to analyze the data of current study. The relationships between studied variables were considered as statistically significant at P- value ≤ 0.05 .

The strength of the associations between studied variables was measured by calculating Odds ratios (ORs) and confidence intervals (95% CI). The categories for OR include greater than 1 and less than 1, in which a value greater than 1 indicates positive association and a value less than 1 indicates negative association.

Results

Patients characterization : A total 85 cases of breast cancer patients were included in this study ,The patients' characteristics are summarized in (**Table-1**). The mean age of breast cancer patients was (44.5) with a range of 25 to 81 years and 57(67.1%) cases were below the age of fifty years, while the 28(32.9%) cases were more than 51-years. Among 85 cases were 78(91.8%) cases grade III and 7(8.2%) were grade II while 41.2%, 25.9% and 3.5% were III ,II and I stage respectively.

Most of our patients in this study(97.6%)were female and only 2.4% were male. We also documented 41(48.2%) of mastectomized cases were lymph node positive while negative lymph node involvement was recorded in 19 (22.4%). Invasive ductal carcinoma was (82.3%) of patients ,while invasive lobular carcinoma and medullary carcinoma were (5.9%)and (11.8%) respectively.

In this study , most of the patients' tumor included were of tumor size $>5\text{cm}$ (67.2%). in addition, our result revealed left sided breast cancer is more than right sided breast cancer.

Immunohistochemical Analysis of PTEN Protein: The immunohistochemical expression of PTEN protein decreased in 50(58.8%) of 85 breast cancer tumors, while PTEN protein expression was normal in 35 (41.2%) of 85 tumors. Our results revealed that expression of PTEN protein was cytoplasmic and/ or nuclear expression of the tumor cells as well as normal ductal epithelial cells and myoepithelial cells were useful as internal positive controls(**Fig-1**).



The relationships between PTEN protein expression and clinicopathological factors (gender of patient, stage, grade, tumor size, tumor site and histological types) in the 85 tumors are shown in **Table (2)**.

There was a significant correlation between PTEN protein expression and lymph node status and age patient while PTEN protein expression did not correlated with gender patient, tumor size, stage, grade, tumor site and histological types table (2). However, loss of PTEN expression increase with high grade and stage as well as large tumor sizes compare with low grade and stage (**Fig-1**).

Table.1.Characteristic Clinic pathological of breast cancer patients.

Parameters		Number	Percentage	Total
Gender	Male	2	2.4%	85
	Female	83	97.6%	
Age	<50	57	67.1%	85
	≥50	28	32.9%	
Grade	I	0	0%	85
	II	7	8.2%	
	III	78	91.8%	
Stage	I	3	3.5%	85
	II	22	25.9%	
	III	35	41.2%	
	Unknown	25	29.4%	
Lymph node status	Positive	41	48.2%	85
	Negative	19	22.4%	
	Unknown	25	29.4%	
Tumor sizes	<2 cm	14	16.4%	85
	3-5 cm	14	16.4%	
	>5 cm	57	67.2%	
Histological types	Ductal	70	82.3%	85
	Lobular	5	5.9%	
	Medullary	10	11.8%	
Tumor site	Left	46	54.1%	85
	Right	39	45.9%	

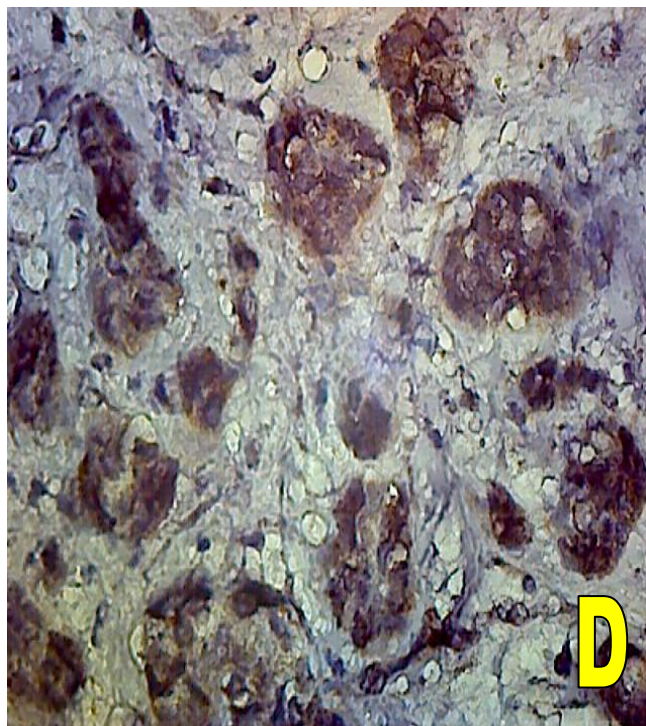
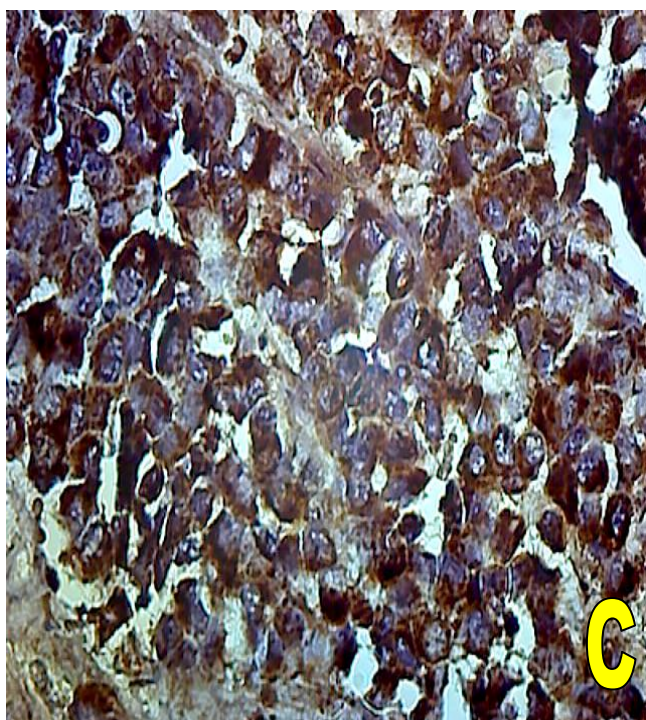
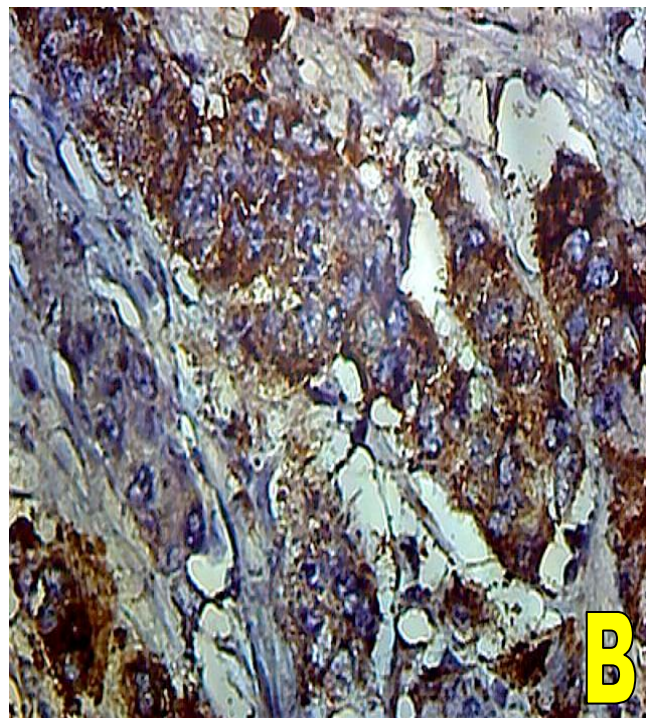
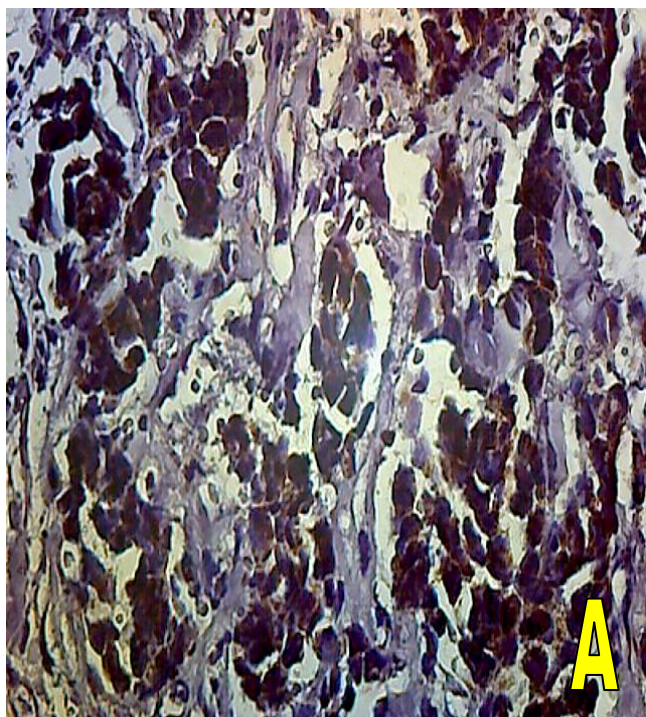


Table.2:Correlation between histopathological parameters and PTEN expression in 85 breast cancer patients.

Parameter		Total	PTEN expression						P value
			Negative			Positive			
Gender		85	0	1	Total	2	3	Total	P value =0.657 O.R = 0.694 95CI=0.042-11.4
	Male	2	0 0%	1 50%	1 50%	1 50%	0 0%	1 50%	
	Female	83	19 22.9%	30 36.1 %	49 59%	18 21.7 %	16 19.3 %	34 41%	
Age patient	<50	57	12 21.1%	16 28%	28 49.1%	17 29.8 %	12 21.1 %	29 50.9 %	P value =0.008 O.R = 0.263
	≥50	28	7 25%	15 53.6 %	22 78.6%	2 7.1 %	4 14.3 %	6 21.4 %	
Histological Types	Ductal	70	16 22.9%	24 34.3 %	40 57.1%	16 22.9 %	14 20%	30 42.9	P value =0.435 O.R = 95CI=
	Lobular	5	1 20%	1 20%	2 40%	2 40%	1 20%	3 60%	
	medullary	10	2 20%	6 60%	8 80%	1 10%	1 10%	2 20%	
Grade	I	0	0 0%	0 0%	0 0%	0 0%	0 0%	0 0%	P value =0.098 O.R = .250 95CI=0.046-1.3
	II	7	2 28.6%	0 0%	2 28.6%	4 57.1 %	1 14.3 %	5 71.4 %	
	III	78	17 21.8%	31 39.6 %	48 61.6%	15 19.2 %	15 19.2 %	30 38.4	
TNM stage	I	3	1 33.3%	0 0%	1 33.3%	2 66.7 %	0 0%	2 66.7 %	P value =0.225 O.R = 95CI=
	II	22	7 31.8%	6 27.3 %	13 59.1%	6 27.3 %	3 13.6 %	9 40.9 %	
	III	35	8 22.9%	18 51.4 %	26 74.3%	5 14.3 %	4 11.4 %	9 25.7 %	
	unknow	25	3	7	10	6	9	15	



	n		12%	28%	40%	24%	36%	60%	
Tumor site	Left	46	13 28.3%	14 30.4 %	27 58.7%	10 21.7 %	9 19.6 %	19 41.3 %	P value =0.578 O.R = 0.989 95CI=0.4 15-2.3
	Right	39	6 15.4%	17 43.6	23 59%	9 23.1 %	7 17.9 %	16 41%	
Lymph node status	Positive	41	8 19.5%	23 56.1 %	31 75.6%	5 12.2 %	5 12.2 %	10 24.4 %	P value =0.032 O.R = 3.4 95CI=1.0 9-10.8
	Negative	19	8 42.1%	1 5.3 %	9 47.4%	8 42.1 %	2 10.5 %	10 52.6 %	
	unknown	25	3 12%	7 28%	10 40%	6 24%	9 36%	15 60%	
Tumor size	≤2	14	3 21.4%	2 14.3 %	5 35.7%	5 35.7 %	4 28.6 %	9 64.3 %	P value =0.137 O.R = 95CI=
	3-5	14	2 14.3%	6 42.9 %	8 57.2	1 7.1 %	5 35.7 %	6 42.8 %	
	≥5	57	14 24.6%	23 40.3 %	37 64.9%	13 22.8 %	7 12.3 %	20 35.1 %	



Figure(1) immunostaining for PTEN in breast tissues, Invasive ductal carcinoma.(A) poorly differentiated (Grade III)Tumor cells show strong nuclear staining.(B) poorly differentiated (Grade III)Tumor cells show strong cytoplasmic staining.(C) poorly differentiated (Grade III) Tumor cells show strong both cytoplasmic and nuclear staining.(D)Moderately differentiated (Grade II))Tumor cells show moderate nuclear staining (40X).



Discussion

PTEN is tumor suppressor gene that appears to have multifunctional role, it play a significant role not only in cell cycle regulation and apoptosis, but also in cell adhesion, migration and differentiation. Recent studies demonstrate that abnormalities in PTEN may be one of the most frequent genetic events observed in human cancers such as breast cancer, and the PTEN expression was reported reduced or absent in 33–50% of breast cancer cases(16,18 and 20). However, The current our study is consider the first of its kind that dealt with patterns of PTEN expression in Iraqi breast cancer patients.

The results observed loss of PTEN expression in 50 (58.8%) of 85 breast cancer tumors, which is a similar result to that of other researchers **Tsutsu et al** (2005) (21) , **Park et al**, (2004) (19) and **chang et al**,(2005) (20) who recorded loss of PTEN expression were found in 28% ,36.5% and 48% of breast cancer tumors respectively.

The loss PTEN expression was more frequency in lymph node positive breast cancer than in lymph node negative breast cancer with significant difference between these two groups($P=0.032$). which is a similar result to that of other researchers **Chung et al**,(2004) (22), **Chang et al**,(2005) (20) and **Tsutsu et al** (2005) (21) who reported the reduced expression of PTEN protein was significant correlated with lymph node metastasis in the breast cancer patients. our explanation for this results probably related with mutation in pten gene as well as other mechanisms such as promoter methylation, translational and post-translational regulation may also play important role on the expression silencing of PTEN protein, While **Zhang et al**,(2013)(23)demonstrated no correlation was observed between PTEN expression and the presence of lymph node metastasis.

We also noticed demonstrated PTEN expression was reduced in 28.6% in grade II compare with 61.5% in grade III and in 33.%, 59% and 74.3% of stage I,II and III respectively. The frequency of reduced expression was highest in advanced breast cancers, with significantly correlated with stage and grade ($p=0.225$ and 0.098 respectively). This results agree with previous studies on breast cancer(18 and 19). Who found no significant difference between stage and grade and loss PTEN expression in breast cancer case. But differs from **Chang et al**,(2005) (20) who reported signifigation correlation between stage and loss PTEN expression.

In our study, there is higher reduce of PTEN expression among those with an age above or equal to 50 years (78.6%) while 49.1% in age group below 50 years old, with signifigation association between reduce PTEN expression and age of the patients($p=0.008$)Table (4.3). This result differed from demonstrated by Park et al, (2004), Chang et al.,(2005) (20) and Zhang et al.,(2013) (23)who found no signifigation association between reduce PTEN expression and age of the patients. our explanation for this results probably related with increasing accumulation mutation in PTEN with age.

Furthermore, The highest percentage of loss PTEN expression cases was observed in the tumor size range $>5\text{cm}$ 65% compare to 57.1%, and 35.7% of tumor sizes 3-5 cm and tumor sizes $<2\text{ cm}$ respectively. It looks that the loss of pten expression is increased with the increase of tumor size without significant difference among these groups. The Same finding was reported by **Chung et al**,(2004) (22), **Tsutsu et al**, (2005) (21) and **Yang et al**,(2010) (15) who showed no signifigation difference between tumor size and loss PTEN expression, in contrasted **Chang et al**,(2005) (20) who suggested near-correlation with the tumor size. while **Zhang et al**,(2013)(23) demonstrated correlation was observed between PTEN expression and tumor sizes,

indicating that the reduced expression of PTEN was associated with a larger tumor size.

Conclusion : These results demonstrated the loss of PTEN expression was found in more than half of breast cancer cases and the reduced expression of PTEN protein was correlated with lymph node metastases and may play a role in progression of breast carcinoma , and hence , worse prognosis in the patients with breast cancer. Beside, the detection of the loss of PTEN expression may serve as a useful biologic marker for progression in invasive breast cancer.

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