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RESEARCH ARTICLE

High Spectrum of PTEN Gene Mutations in Iraqi Breast Cancer Patients

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Abstract

Breast cancer conceder the higher tumor rate among Iraqi women. New potential and prognostic markers are required to identify those women. *PTEN* gene (phosphatase and tensin homolog deleted from chromosome 10) is tumor suppressor; its mutations in breast cancer are well reported in many populations but not in the Iraqi people. The main purpose of this research was to identify the *PTEN* mutation spectrum as predictive marker in Iraqi patients with breast cancer and benign lesion. The mutations in the 7th exon of *PTEN* gene was analyzed on thirty tow Iraqi females with breast disease, twenty women were diagnosed breast cancer and six women with precursor lesions of the breast (benign) in addition to six healthy women served as reference (controls). Results showed several mutations including thirty different mutations in malignant samples, the most five were characterized by high alteration rates75% (15/20), at position 115833 and 115950 these are T>G and T>A followed by 65%(13/20) and 50 % (10 /20) at positions 115921 and 115843 respectively this is G>C, other more mutations frequency 45% (9/ 22) at position 115912 this is G>T .We also detected an insertion G and T between (115833-115834) in 20%(4/20) and 10 % (2/20) respectively .Comparison of mutations between malignant and benign patients showed the frequency of several mutations in three sits (115833, 115843 and 115912) were appear in both benign and malignant patients may indicating to important role of the three sits in initiation and progression of breast cancer. Other two positions (115832, 115949) also have high mutations rate were found only in malignant patients indicating that these positions may have a role in progression of breast cancer. In conclusion we believe that *PTEN* gene is a promising biomarker that could be useful in detection of breast cancer patients and predicting the prognostic state of those women.

Keywords: *Iraq, PETN gene, Breast cancer.*

Introduction

The National Cancer Institute in America had been published that were more than 1.65 million new cases of breast cancer and 522,000 deaths in 2012 [1]. It is represented the most important neoplasm among women in the majority of the developed countries, accounting for one-third of recently identified malignancies [2].

In 2012 had been published that 4,115 cases of breast cancer were reported by Iraqi Cancer Registry accounting for 19.5% of all recently identify malignancies , 34% of the registered female breast cancers, with an incidence approximating twenty two per 100,000 female population [3].

It is though that breast cancer is extremely heterogeneous disease, comprise a number of biologically different entities with specific pathologic character and biological action [4].

Breast cancer include a series of steps: initiating atypical ductal hyperplasia, followed by ductal carcinoma in situ, subsequent development to culminating as invasive ductal carcinoma, lastly progress to metastatic disease, it's a stepwise accumulation of many genetic changes leading to activation of oncogene or inactivation of tumor suppressor genes [5].

Several pathways have important role in the development, classification and prognosis of breast cancer like PI3K/PTEN/Akt/mTORC1, patients show mutations or changes in the expression profile of these pathways [6]. PI3K mutations cause deregulation of its pathway in breast cancer and most frequently happen through mutations in catalytic subunit alpha of phosphatidylinositol -4,-5 bisphosphate 3-kinase (*PIK3CA*) [7].

PTEN gene (phosphatase and tensin homolog) is tumor suppressor gene which encodes a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase that neutralize the action of the *PI3K* gene, inactivation of *PTEN* gene also leads to transmit signals of growth factors from receptor tyrosine kinases to downstream mediators such as the AKT family of serine/threonine-specific protein kinases [8] , thus encourage cell growth and proliferation finally survival and migration through multiple downstream effectors [9].

The important role of *PTEN* gene induction of apoptosis [10, 11].*PTEN* gene is mutated or silenced in many tumor types [12] melanoma, pancreatic cancer, colorectal cancer, lung cancer and leukemia [13].

It is play great role in promoting and development of breast cancer through several mechanisms, including, germline and somatic mutations in the *PTEN* gene, loss of heterozygosity at the *PTEN* gene locus, methylation of the *PTEN* promoter lead to epigenetic silencing, down-regulating PTEN transcription results from protein interactions, [14, 15].

Considering the effect of *PTEN* gene in breast cancer initiation, development, and prognosis, we investigated the existence of mutations in exons 7 as biomarkers in breast disease Iraq females and comparison the results with *PTEN gene* polymorphism of worldwide populations.

Materials and Methods

The study was performed on twenty six females with breast disease; twenty women were diagnosed with invasive or *in situ* ductal breast cancer and six women with precursor lesions of the breast (benign) in addition to six blood samples from healthy women served as reference (controls).

Tissue samples were collected from private laboratories in Baghdad city between July, 2016 and February, 2017, it was store by freezing until DNA extraction. The diagnosis and selection of patients were assessed under the supervision of pathologist committee.

Genomic DNA was extracted from breast cancer tissue samples using the QIAamp DNA Mini kit (Qiagen), Hilden, Germany.

Polymerase Chain Reaction (PCR)

PCR was performed to amplify the *PTEN* gene. The primer pairs located in exon 7 of the *PTEN* gene, forward primer was 5´TTGACAGTTAAAGGCATTTC-3´ and the reverse primer was 5´-CCTATTTTGGATATTTCTCCC-3 [16].

The PCR reactions were achieved through a former denaturation at 94°C for 10 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 51°C for 30s, and 72°C for 30s, after that extension at 72°C for 10 min followed by a final extension at 72°C for 10 min and hold at 4°C.

PCR reactions were done in 25µl reaction mixture for PTEN amplification include 12.5 µl of master mix and 1.5 µl of each primer and 9.5 µl of genomic DNA PCR amplifications were achieved in an Applied Biosystem 96 thermo cycle.

Sequencing

All DNA templates were sent to Micro gene Company (Koria). It was processed for the DNA sequencing reaction. Each sample was amplified in a new 25 µl PCR reaction and sequenced using the same forward primer.

Results and Discussion

Patients and Disease

Using genomic DNA, isolated from tissue samples of breast disease patients and peripheral blood healthy individuals. Twenty-six breast disease patients were investigated, including 20 samples with breast cancer and 6 benign samples in addition to six healthy blood samples. The mean age of patients was 50 years the average age was (38-62) years, Patient characteristics are shown in Table 1.

Table 1: The distribution of patients with breast cancer

Characterization	Total no. (%)
All patients	20 (100)
Age yrs	
Average age	48
> 50	5(25)
≤ 50	15(75)
Gender	
Female	20(100)
Site of tumor	
Right breast	8(36.3)
Left breast	6(27.2)
Bilateral breast	1(4.5)
Axillary	7 (32)
Differentiation	
Moderately	14(64)
Well	2(9)
Poorly	6(27)

Mutations Analysis in Exon 7 of PTEN Gene

Breast cancer conceder the higher tumor rate among Iraqi women and it is diagnosed in late stage among middle-aged Iraqi women leading to death with short survival making it a more public health defy in Iraq [17, 18]. Therefore, it is important to study potential biomarkers that may help to screen the patients at high-risk and to predict the prognosis breast cancer. New potential and prognostic markers are required to identify those women.

PTEN mutations in breast cancer are well reported in many populations [19, 20] but not in the Iraqi people. The main purpose of this research was to identify the *PTEN* mutation spectrum as predictive marker in Iraqi patients with breast cancer and benign lesion. PTEN protein consists of two domains N-terminal phosphatase and a Carboxyl terminal (C2) domains, the phosphatase domain responsible for the enzymatic activity of the protein, while the carboxyl domain binds the membrane phospholipids, Exon 7 in *PTEN* gene encodes for calcium binding region 3 (CBR3) loop which is important for binding of the C-terminal domain with the rest of the protein [21]. Several mutations was appear in present study including thirty different mutations were found in exon 7 when comparing all sequence variants with NCBI data base accession no. AF067844, Table (2) show the most detected alterations, five were characterized by high alteration rates75% (15/20), at position 115833 and 115950 these are T>G and T>A followed by 65%(13/20) and 50% (10/20) at positions 115921 and 115843 respectively this is G>C, other more mutations frequency 45 % (9/22) at position 115912 this is G>T.

We also detected an insertion G and T between (115833-115834) in 20 % (4/20) and 10 % (2/20) respectively. Other single nucleotide polymorphism were neglected because it's not frequency in more than one sample (Figure 1), but the most interesting thing is the presence of G>A mutation was showed in all including healthy samples at (115855, 115952, 115969 and 115975) positions it may explain by specific genetic variation in Iraqi people.

Table 2: Totally of mutations in the 7th exon of *PTEN* gene in 20 malignant samples

Mutations	Number of samples	%	Position
T>C	3	15	115825
Del A	1	5	115827
A>C	1	5	115827
A>G	1	5	115827
G>T	4	20	115828
G>A	1	5	115828
G>T	5	25	115832
T>G	15	75	115833
Ins G	4	20	115833-115834
Ins T	2	10	115833-115834
T>C	4	20	115836
T>G	5	25	115838
G>C	10	50	115843
T>A	2	10	115846
T>C	3	15	115865
A>C	2	20	115882
G>A	2	20	115889
G>T	9	45	115897

A>C	2	10	115905
G>T	9	45	115912
T>C	4	20	115918
G>C	13	65	115921
T>G	4	20	115930
T>G	2	10	115939
G>T	5	25	115949
T>A	15	75	115950
G>T	7	35	115954
A>C	4	20	115962

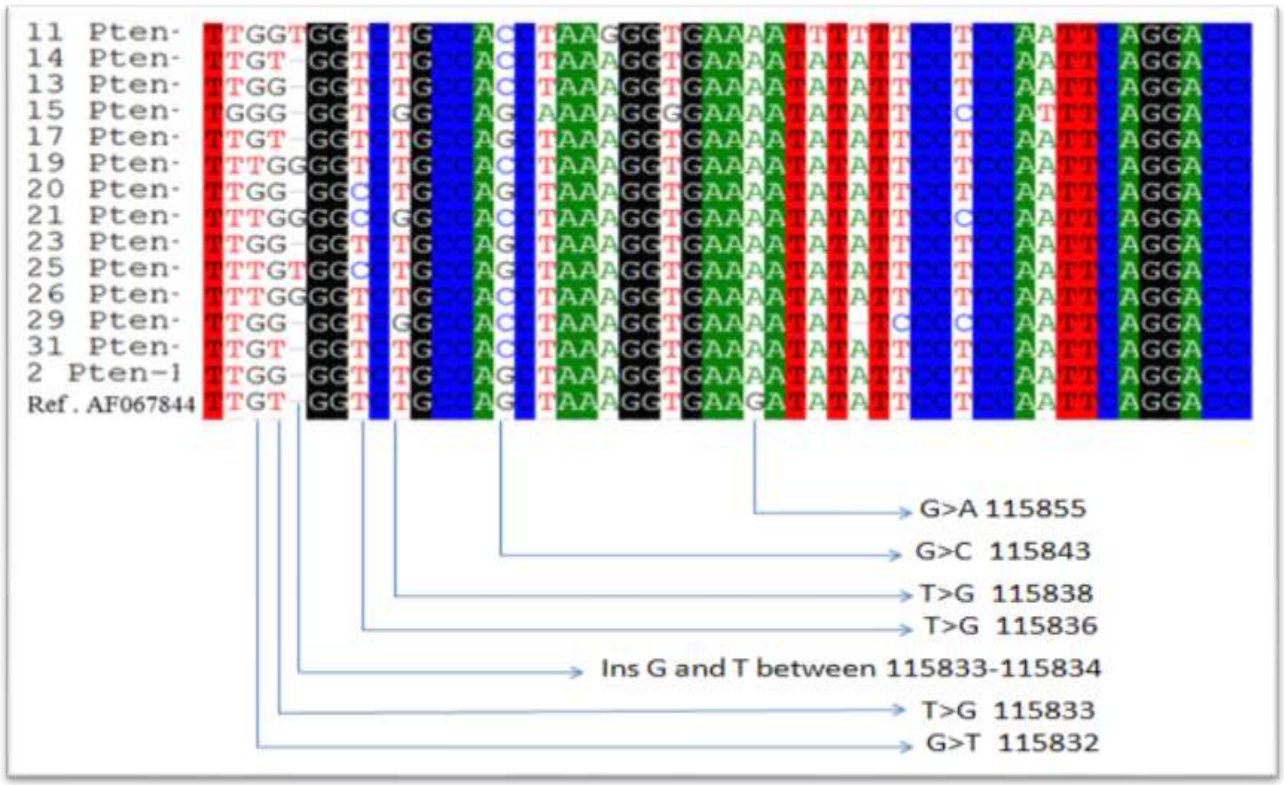

Figure 1: Representative data of sequences analysis of the *PTEN* gene in some malignant breast samples in comparable with Ref. AF067844, arrows indicate to mutations sites

Table (3) showed mutations in the 7th exon of PTEN gene in benign lesion samples, most of the them were similar to that were found in malignant samples. Comparison of mutations between malignant and benign patients in Table (4), showed the most mutations (5) Ins G and (2) Ins T between (115833-115834) sites were appear in malignant samples while (4) T>G mutations in 115833 positions in benign samples, also G>C mutation was appear in (10/20) and (4/6) in malignant and benign samples respectively in 115843 position in addition to G>T mutation was showed in (9/20) and (4/6) in malignant and benign respectively in 115912 position, the frequency of this alterations in both benign and malignant samples may indicating to important role of the three sits (115833, 115843 and 115912) in initiation and progression of breast cancer. Other mutations were appear only in malignant samples including G>T mutations in two positions (115832, 115949) with rate 25% (5/20) and A>C mutations in 115962 with 20% (4/20) indicating that these positions may have a role in progression of breast cancer.

Table 3: Totally *PTEN* gene mutations in benign samples

Sample Number	Mutations	Position
4	G>T	115828
4	T>G	115833
2	T>C	115836
2	T>G	115838
6	G>C	115843
2	DelA	115858
6	G>T	115897
4	G>T	115912
6	G>C	115921
2	T>G	115939

The results of present study showed high rate of *PTEN* gene mutations more than those were found in our previous published work of molecular analysis of *PTEN* gene in Iraqi colorectal cancer patients [22]. This may due to hormonal activity that considered a major factor in initiation and progression of breast cancer by three postulated mechanisms to be induces their carcinogenic effects first, cellular proliferation that stimulation by hormones ,second increasing rates of mutations as direct genotoxic effects through mitochondrial P450-mediated metabolic activation finally induction of chromosomal aberration [23]. Important work was done by Kechagioglou *et al*., to investigate the *PTEN* gene mutations, heterozygosity in addition to protein expression in benign and malignant of the breast, their results indicate that breast cancer is linked with decreased heterozygosity and several mutations were found in the *PTEN* gene but they couldn't found relation with loss of PTEN protein expression, breast malignant appears to be associated with a high rate of mutations that inactivate its corresponding protein, they concluding a possible mechanism activity of PTEN loss in breast cancer related to frequency of gene mutations in three exons (1,7and 9) of the *PTEN* gene may be lead to increase phosphorylation of PTEN protein in breast cancer [24]. Alterations in the *PTEN* gene are involved with loss of activity even though its coded protein is expressed [25].

Table 4: Comparison of mutations between benign and malignant patients

Type of mass	mutation	No. samples	Position of mutation
Malignant	Del A	(1)	115827
Malignant	A>G	(1)	
Malignant	A>C	(1)	
Benign	-	-	
Malignant	G>T	(5)	115832
Benign	-	-	
Malignant	Ins G	(4)	115833-115834
	Ins T	(2)	
Benign	T>G	4	115833
Malignant	G>C	10	115843
Benign		4	
Malignnt	T>A	(2)	115845
Benign	-	-	
Malignant	T>C	(3)	115865
Benign	T>C	(1)	
Malignant	G>T	(9)	115912
Benign		(4)	
Malignant	T>C	(4)	115918
Benign	T>C	(1)	
Malignant	G>T	(5)	115949
Benign	-	-	
Malignant	G>T	(7)	115954
Benign	G>T	(2)	
Malignant	A>C	(4)	115962
Benign	-	-	

Recently PTEN loss rate also have been shown in breast cancer, it was significantly higher than that in normal tissues [26]. Loss of PTEN activity (PTEN was lost in 30 % primary tumors and 25 % metastases) cause increasing the phosphorylation of P13K pathway which leads to breast malignant [27].Other study from Pakistan showed that genetic variations in *PTEN* tumor suppressor gene were related with breast cancer [28].

Yang *et al*., investigated the carcinogenesis role of *PTEN* mutation in breast by screening its mutation spectrum and expression of corresponding protein in Yunnan and China region, high rate of *PTEN* mutations were found at the early stage development of breast cancer and associated with expression silencing of its protein concluded the significant role of *PTEN* gene mutation in breast cancer carcinogenesis as a results of variations in exon seven which coded for the CBR3 loop of PTEN protein lead to completely disrupt C-terminal domain association with the rest of PTEN protein [29]. Other genetic changes in *PTEN* gene such as deletions, reduced RNA or protein levels due to transcriptional disregulation from epigenetic down-modulation of PTEN or increased protein degradation due to increased ubiquitination would be expected to be identified by immunohistochemical since these are

ultimately mediated through reduced protein expression [30].

In Saudi Arabia the promoter methylation and loss of expression of *PTEN* gene in breast malignant patients were analyzed to investigate the correlation between methylation and gene expression, the authors found promoter methylation and lack of PTEN protein occur frequently in those patients suggesting that methylation of *PTEN* gene have important role in carcinogenesis of breast cancer [31]. Golmohammadi et. al., from Iran also referred to that loss of PTEN gene expression may be indicate to a worse prognosis causing poor survival in breast malignant patients [32].

In recent article the significance of PTEN expression in breast cancer was evaluated in 27 studies from different countries (USA, U K, Italy, Germany Korea, Turkish, Iran, Saudi Arabia, China , and other) involving 10,231 patients, the pooled conclusions revealed that PTEN loss was significantly with high rates in breast cancer than in normal, it may be act as predictive marker for more aggressive behavior tissue , it was found clear correlation of clinical characteristic like larger tumor size and lymph node metastatic with loss of PTEN expression suggestion worse outcomes in patients with this disease ,as mention above mutation in *PTEN* gene is one of the PTEN loss causes and that might predict more aggressive behavior and worse outcomes in patients with breast cancer [33].

Taking our study's results into consideration, we believe that *PTEN* gene is a promising biomarker that could be useful in detection of breast cancer patients and predicting the prognostic state of those women. Complementing our finding with further studies in larger patient's groups are required to investigate the association between *PTEN* gene and clinicopathological parameter may provide more clear picture about its role in carcinogenesis of breast.

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