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Mutational changes in two randomly selected regions (exon 3 and exon 7) of estrogen receptor-beta gene in Iraqi breast cancer female patients

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Abstract

The study investigated mutational changes in exon 3 and exon 7 of estrogen receptor-β (*ER-β*) gene in breast tumor (benign and malignant) of Iraqi female patients; each with 30 participants, who were referred to the Center for Early Detection of Breast Tumor at Al-Alwayia Hospital for Gynecology and Obstetrics (Baghdad) during the period June-December 2011. In addition, 30 female controls were also enrolled in the study. Conventional PCR analysis of *ER-β* gene for specific coding sequences in exons 3 and 7 revealed that 20.0% malignant breast tumor patients did not show the band (PCR amplified product) of exon 3 after agarose-gel electrophoresis, while the rest of patients and controls showed the band. For exon 7, 80.0% of malignant breast tumor patients and 23.3% benign breast tumor patients did not show the band, while all women of controls (100%) showed the band. These differences were highly significant with a probability range of 0.01 - 1.7 x 10^{-11}; therefore mutational changes in *ER-β* are of importance in etiology of breast cancer.

Key words: Estrogen receptor-β gene, Breast cancer, Iraq.

Introduction

The burden of breast cancer is increasing in both developed and developing countries, and in many regions of the world, it is the most frequently occurring malignant disease in women, comprising 18% of all female cancers, and worldwide, breast cancer is the fifth most common cause of cancer mortality (Bray *et al*., 2012). In 2008, approximately 1.4 million women were diagnosed with breast cancer worldwide with a corresponding of 460,000 deaths (Ferlay *et al*., 2010). In Iraq, breast cancer is the commonest type of female malignancy, accounting for approximately one-third of the registered female cancers according to the latest Iraqi Cancer Registry (Iraqi Cancer Registry, 2010), and is the second cause of cancer related deaths (Saaed *et al*., 2011).

No specific etiological factor has been documented, but different breast cancer-associated risk factors have been suggested by epidemiological studies; for instance, age, menarche, menopause, parity, breastfeeding, use of exogenous hormones or oral contraceptive, obesity, lack of exercise, diet, cigarette smoking, alcohol consumption and family history of breast cancer or other cancers (Davies, 2012). However, these risk factors have been shown to have different relations to breast cancer in different ethnic populations of the world (Abdulrahman and Rahman, 2012). Accordingly, breast cancer is clinically regarded as a heterogeneous and complex disease, encompassing a wide variety of pathological entities and a range of clinical behavior. This heterogeneity is strictly linked to individuals and tumors genetic variability, therefore it is now widely accepted that accumulation of genetic anomalies contributes to the acquisition of an increasingly invasive or chemo-resistant tumor phenotype (Cavallaro *et al*., 2012).

It is well-established that breast cancer typically arises in luminal epithelial cells of the mammary gland, and these cells contain estrogen receptors (ERs), which respond to ovarian estrogen in normal mammary gland development. How estrogens stimulate cell growth is not fully understood, but it is known that estrogen activation of ERs results in transcription of various genes that are involved in cellular proliferation (Althuis *et al*., 2004). However, only a small fraction (< 5%) of women diagnosed with breast cancer has a clear hereditary predisposition, and of these, about one half has predisposing mutations in *BRCA1*, *BRCA2* and *TP53* genes, or other known cancer predisposing genes (Yager and Davidson, 2006), but twin studies have indicated that

the heritability of breast cancer is about 30%, suggesting that genes other than the well-mapped regions act as modifiers of breast cancer risk (i.e. *ER-α* and *ER-β* genes) (Abbasi, 2010). Estrogen receptor-α polymorphic variants have been associated with breast cancer risk in Caucasians, but supporting evidence is required for proving the involvement of ER-β in breast cancer, and currently, the literature contains sparse information regarding *ER-β* gene expression, mutation frequency, and allelic variation in breast cancer (Abbasi *et al*., 2012).

Accordingly, the present study selected two coding regions (exon 3 and exon 7) of the *ER-β* gene to detect possible mutational changes that might be associated with risk of breast cancer in Iraqi female patients. Such selection was based on the findings of Abbasi (2010), who scanned these two exons and suggested that might have been involved (especially exon 7) in various aspects of breast cancer and lymph node metastasis in a group of Iranian patients.

Materials and Methods

Investigated subjects: The study involved 90 women, who were distributed into two groups of patients and a group of controls. The patients were women who had a breast tumor, and according to the type of tumor, they were distributed as malignant and benign groups, each with 30 patients. Malignant tumor group included patients whose age range was between 30 and 65 years (mean ± standard error: 49.8 ± 1.7 year), while such range in benign tumor group was 21-60 years (39.5 ± 1.9 year). The patients were referred to the Center for Early Detection of Breast Tumor at Al-Alwayia Hospital for Gynecology and Obstetrics (Baghdad) during the period June-December 2011. The diagnosis was made by the consultant medical staff, which was based on a Triple Assessment Technique (i.e. physical breast examination, ultrasonography, with or without mammography and fine needle aspiration cytology). In addition, 30 female controls were also enrolled in the study, and their age range was 18-64 year (35.6 ± 1.8 year), and they were ethnically matched with breast tumor patients (Iraqi Arabs).

Blood collection and PCR analysis: From each participating women, 2 ml of venous blood were collected and dispensed in EDTA tube and frozen at -20°C until PCR analysis of *ER-β* gene; from which, the genomic DNA was extracted using the ReliaPrep™ Blood gDNA Miniprep System (Promega Corporation, USA), which is designed to provide a fast and simple technique for the preparation of purified and intact DNA from mammalian blood. The DNA yield was spectro-photometrically assessed using Cecil E1021 spectrophotometer (England), in which the sample was read at two optical densities that were 260 and 280 nm. The first reading was divided by the second reading, and if the outcome was 1.8-2.0, the sample was considered as free of contamination and having a sufficient amount of DNA for a further analysis. The DNA concentration was calculated using the formula that was given by Sambrook *et al*. (1989).

Based on the findings of Abbasi (2010), exons 3 and 7 of *ER-β* gene were selected. The author designed two specific set of primers for the two exons of *ER-β* gene, using *primer3* (version 0.4.0) software (Table 1). The PCR amplification was carried out in a total volume of 25 µl consisting of 1U of Taq DNA polymerase, 1× Taq DNA polymerase buffer, 1 µl extracted DNA, 200 µM deoxyribonucleotide triphosphate, 1 mM $MgCl_2$, and 0.8 µM of primer. Amplification was performed with an initial denaturation step at 95°C for 7 minutes, followed by 35 cycles of: denaturation at 95°C for 45 seconds, annealing at 53°C for 1 minute, extension at 72ºC for 1 minute, and a final extension step at 72ºC for 7 minutes. (Safarinejad *et al*., 2010). The amplified PCR fragments were accomplished by electrophoresis on agarose gel, and then amplified DNA sequences were visualized as bands by UV illumination after ethidium bromide staining. If the amplified PCR product is intact and there is no change in its sequence (no mutation or mutations), then the band is present; otherwise, no band can be visualized if there is any change in the sequence and a mutation or mutations can be pictured.

Statistical analysis: Significant differences between investigated groups were assessed by two-sided Fisher's exact probability (P), by using the statistical package PEPI version 4.

Table 1: The selected primers for exons 3 and 7 of *ER-β* gene*.

Exon	Melting Temperature (°C)	Oligonucleotic Sequences	Sequence size
Exon 3	59.23	**F** 5'-TTGCTCCCTAGAGAGACACTGA-3'	151
	59.86	**R** 5'-CTTCACACGACCAGACTCCA-3'	
Exon 7	60.04	**F** 5'-GATGAGGGGAAATGCGTAGA-3'	156
	60.14	**R** 5'-GGCCCAGCTGTGTGATTACT-3'	

***After Abbasi (2010).**

Results and Discussion

For exon 3, only six malignant breast tumor (BT) patients (20.0%) did not show the band, while all benign BT patients and controls (100%) showed the band (Figure 1 and Table 2). Such difference was significant (P = 0.01). In case of exon 7, the outcome was different, in which 24 of malignant BT patients (80.0%) and seven of benign BT patients (23.3%) did not show the band, while all women of controls (100%) showed the band (Figure 2 and Table 2). Such difference was highly significant (P = 1.7×10^{-11}, 1.7×10^{-5} and 0.005; for the comparisons: malignant BT *vs*. controls, malignant BT *vs*. benign BT and benign BT *vs*. controls, respectively). These results demonstrate that mutational changes occurred in *ER-β* gene of BT patients, and most of these mutations were observed in exon 7 of the gene of malignant BT patients; and accordingly, such gene defect(s) might have its effect on the progression of breast cancer in the investigated patients. However, the investigator was unable to go further to detect these mutations because of the limited technical resources, and certainly future studies in Iraq can highlight this matter that might have importance from the point views of prognosis and treatment. This is related to the fact that genetic variations in genes involved in estrogen synthesis, metabolism and signal transduction have been suggested to play a role, by influencing the growth, differentiation and function of breast tissue، and exerting their biological effect through binding to ERs (Tsezou *et al*., 2008).

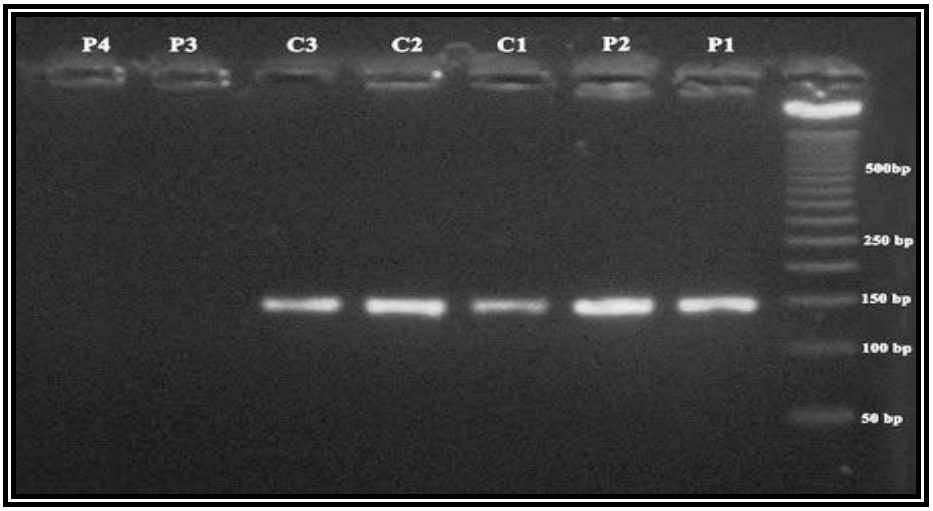

Figure 1: Agarose gel electrophersis of *ER-β* gene exon 3 PCR amplified products. P1 and P3: malignant breast tumor patients; P2 and P4: benign breast tumor patients; C1, C2 and C3: controls.

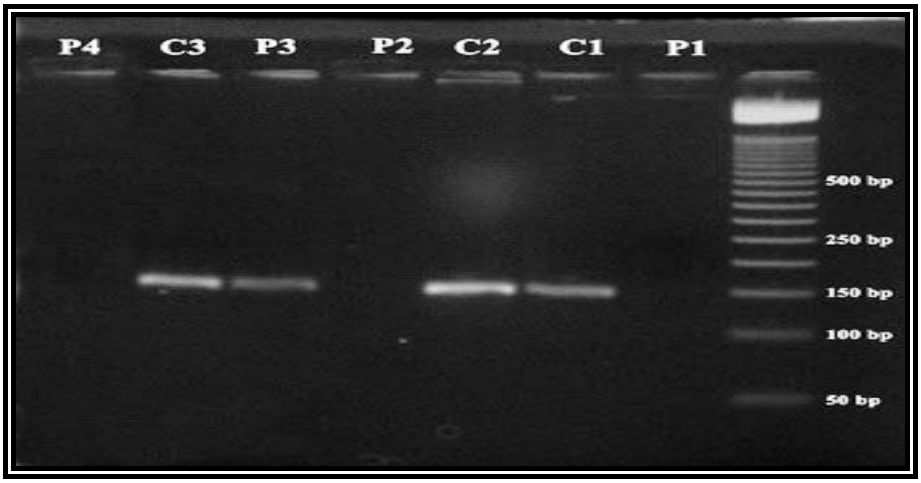

Figure 2: Agarose gel electrophersis of *ER-β* gene exon 7 PCR amplified products. P1 and P2: malignant breast tumor patients; P3 and P4: benign breast tumor patients; C1, C2 and C3: controls.

Table (2): Malignant and benign breast tumor patients and controls characterized by exons 3 and 7 of estrogen receptor-beta gene.

Groups		Number	Estrogen Receptor-Beta Gene*			
			Exon 3		Exon 7	
			No.	%	No.	%
Breast Tumor Patients	Malignant	30	6	20.0	26	80.0
	Benign	30	0	0.0	7	23.3
Control Women		30	0	0.0	0	0.0

***Numbers represent women that did not show bands after agarose-gel electrophersis of *ER-β* gene exons 3 and 7 PCR amplified products.**

There are two subtypes of ER (ER-α and ER-β), which have been demonstrated to have different expression profiles in normal and malignant tissues; an observation that paved the way to the possibility that ER+ve breast tumors might be even more heterogeneous than originally supposed (Fox *et al*., 2008). Estrogen receptor-β is one of the most important determinants in the mechanism of estrogen action and it has been suggested to be an obvious candidate gene to harbor allelic variants that predispose to breast cancer (Abbasi *et al*., 2009). Further studies suggested that gene expression, mutation frequency and allelic variation of *ER-β* gene in breast cancer might be abnormally regulated (Abbasi *et al*., 2012). More recently, Yan *et al*. (2013) provides evidence that allelic variation of ER-β1 could be predictive biomarker of tamoxifen benefit in early breast cancer. These demonstrations, together with the findings of present study suggest the prognostic potential of *ER-β* gene and its mutations in the etiology of breast cancer, and further detailed molecular analysis of these mutations will certainly lead to a fruitful understanding in this regard

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