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Influence Of Oxidative Biomarkers On APE1 Gene Expression In Iraqi Breast Cancer Women

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Abstract

This study aimed to investigate the APE1 gene expression with some clinical biomarkers in breast cancer women. Ninety women were enrolled in this study; they were divided equally into 2 groups: the breast cancer patients' group, which attended the Al-Eluia hospital for women's care in Baghdad, and the healthy control women's group. Five milliliters of blood were taken from patients and a healthy control group. Two milliliters of blood were transmitted directly to EDTA tubes, and then the ELISA technique was used to determine the oxidative biomarkers like Glutathione Peroxidase 1 (GPX1), Estradiol (E2), Malondialdehyde (MDA), and reactive oxygen species (ROS). Three milliliters of blood were used for gene expression by using the RT-PCR method. The result of this study showed that there were significant differences in the expression of the APE1 gene were the gene folding for patients was 3.004 ±0.15 when compared with the control group 1.00 ±0.00. In conclusion, when comparing breast cancer patients to healthy people, it was discovered that the APE1 gene was out of the ordinary.

Keywords: APE1 gene, TFs, Breast cancer, GAPDH, RT-PCR.

Introduction

Breast cancer is the most prevalent cancer-related cause of death among women across the world. Rates differ about five-fold across the globe, but they are rising in regions that have had low disease rates until recently [1]. The rise in cancer incidence in recent years, as well as its influence on various physical, mental, and social aspects of a person, has elevated cancer to a massive issue of the century [2]. In privileged areas, the prevalence of this condition ranges from 1 to 2%, with a nearly 5% annual increase in less developed countries [3]. The most widespread kind of cancer among women is breast cancer. A patient's chance of dying from breast cancer is one in 35 [2]. X-ray mammography, ultrasound, and magnetic resonance imaging (MRI) are the most regularly utilized clinical diagnostic techniques for breast cancer [4]. A kind of fatty tissue called adipose tissue makes up the breast. Female breasts often have more glandular tissue than male breasts [5]. Female breasts contain 12-20 lobes, which are subdivided further into tiny lobules that are connected through milk ducts to these lobes and lobules [6]. The adipose tissue of the breast is full of a network of nerves, blood vessels, lymph vessels, lymph nodes, fibrous connective tissue, and ligaments [6]. Redox effector factor 1, commonly known as Ref-1, works as a reductive stimulator of a variety of transcription factors (TFs). For example, APE1 is involved in apoptosis, inflammation, angiogenesis, and many pathways by

stimulating activator protein-1, nuclear factor kappa B, hypoxia-inducible factor 1, coupled box gene 8, signal transducer activator of transcription 3, as well as p53. Cellular homeostasis is controlled by APE1/Ref-1 (redox) through activating transcription factors (TFs) that govern a variety of physiological processes and interact with redox balancing agents (such as thioredoxin, catalase, and superoxide dismutase) to control reactive oxygen and nitrogen species concentrations. The effectiveness of APE1/function(s) Ref-1's is determined by double-bound interactions with participant protein(s); the BER pathway, TFs, energy metabolism, cytoskeletal elements, and stress-dependent responses are among the activities regulated by APE1/Ref-1. As a result, APE1/Ref-1 functions as a "hub protein" that regulates essential cell survival pathways [7]. The BER process is aided by the apurinic/apyrimidinic endonuclease (APE), APE1 on chromosome 14 (14q11.2–q12), the APE1 gene comprises five exons with a 2.21 kb span [8], when hydrolyzed at the 3′ end, it inhibits DNA oxidation, forming 3′-hydroxyl termini, which is essential for DNA repair upon single- or double-strand breaks [9,10]. This study aimed to investigate the APE1 gene expression with some clinical biomarkers in breast cancer women.

Materials and Methods

This study included 90 participants (45 newly diagnosed breast cancer patients and 45 healthy controls) who ranged in age from 30 to 70 years old. Al-Eluia hospital for woman care, a cancer teaching hospital, provided blood samples. Every patient was given a thorough physical examination. the final diagnosis was made by aspirating cysts and checking cytology, histology (biopsy), and mammography.

The estimation of the APE1 gene expression level:

RNA Extraction

All buffer preparation is made at room temperature (Zymo, U.S. A).

RNA Purification

1. An equal volume of ethanol (95-100%) was added to a sample lysed in TRI Reagent and mixed thoroughly.

2. The mixture was transferred into a Zymo-Spin™ IIC Column in a collection tube and centrifuged at 16,000 rpm for 30 seconds.

3. The column was transferred into a new collection tube and the flow-through was discarded.

4. 400 µl RNA Washing Buffer was added to the column and centrifuged at 16,000 rpm for 30 seconds.

5. In an RNase-free tube, 5µl DNase I (6U/µl), 75µl DNA Digestion Buffer, and mix4. The mix was directly added to the column matrix and incubated at room temperature for 15 minutes.

6. 400 µl of Direct-zol™ RNA Pre Wash was added to the column and centrifuged at16,000 rpm for 30 seconds. The flow-through was discarded and this step was repeated.

7. 700µl of RNA Washing Buffer was added to the column and centrifuged for two minutes at16,000 rpm to ensure complete removal of the wash buffer.

8. The column was carefully transferred into an RNase-free tube.

9. To elute RNA, 50µl of DNase/RNase-Free Water was added directly to the column matrix and centrifuged at 16,000 rpm for 30 seconds.

Reverse Transcription (RNA to cDNA) procedure

Prime Script TM RT reagent Kit is designed to perform the reverse transcription optimized for real-time (RT-PCR).

Protocol:

1. The following reaction mixture was prepared on ice.

2. 2µl from 5 × Prime Script TM mix was added to 3-5 µl total RNA.

3. The volume was completed up to 10 µl. as seen in the table (2-1).

4. The reaction mixture was incubated under the following condition. 37°C for 15 min (Reverse transcription), 85°C for 5 sec, and 4°C to Inactivate reverse transcriptase with heat treatment.

Table (2-1): Components of Reverse Transcription.

Reagents	Volumes
5 × Prime Script TM mix	3-5 µl
total RNA	8 µl
RNase Free dH2O	up to 10 µl

real-time- PCR

The primers (IDT, U.S. A) APE1 Primer sequence (5 /→ 3 /) F: GAGTAAGACGGCCGCAAAGAAAAA

R: CCGAAGGAGCTGACCAGTATTGAT

and GAPDH (Reference gene) Primer sequence (5 /→ 3 /) F: CACTAGGCGCTCACTGTTCTC

R: AATCCGTTGACTCCGACCTT

KAPA SYBR® FAST qPCR Master Mix (2X) Kit (Takara, Korea).

Are a ready-to-use cocktail containing all components (except primers and template) for the amplification and detection of a target in qPCR.

Procedure

1. All reaction components were properly thawed and mixed.

2. A No Template Control (NTC) was included to allow for the detection of contamination of reaction components.

3. 10 µl from KAPA SYBR FAST qPCR Master Mix (2X) Universal was added, and 0.4 µl for each Forward and Reverse primer, and Nuclease-free water Up to 10 µl.

4. The required volume of each component was calculated based on the table (2-2).

5. after the addition of the components the tubes have sealed and placed into a thermal cycler that is programmed as summarized in table (2-3) for the APE-1 gene and in the table (2-4) for GAPDH.

Table (2-2): Reaction components of RT-PCR.

Component	volume (µL)	Final concentration
KAPA SYBR FAST qPCR Master Mix (2X) Universal	10	2x
Forward primer	0.4	0.2µM
Reverse primer	0.4	0.2µM
Nuclease-free water	Up to 10	
Template cDNA Sample Volume	3	1pg-100ng

Table (2-3): RT-PCR Cycling Program.

Steps	Temp. (°C)	Time	Cycle
Enzyme activation	95	5 min	Hold
Denaturation	95	20 sec	40
Annealing	60	20 sec	
Extension	72	20 sec	

Table (2-4): RT-PCR Cycling Program of reference gene (GAPDH).

Step	Temp. (°C)	Time	Cycle
Enzyme activation	95	5 min	Hold
Denaturation	95	20 sec	40
Annealing	56	20 sec	40
Extension	72	20 sec	40
Final extension	72	2 min	1

Gene expression equations

ΔCt=Target Ct- Reference Ct

ΔΔCt=ΔCt (for each sample)-mean ΔCT of normal

Fold change =2- (ΔΔCt) [11].

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors on study parameters. A T-test was used to significantly compare between means. The Chi-square test was used to significantly compare percentages (0.05 and 0.01 probability) [12].

Results and Discussion

The estimation of the APE1 gene expression level

Gene expression of this marker was used using q RT-PCR. All samples were run in duplicate. Amplification plots between the fluorescent signals from each sample against cycle number represent the accumulation of product throughout the real-time PCR experiment as shown in figure (3-1).

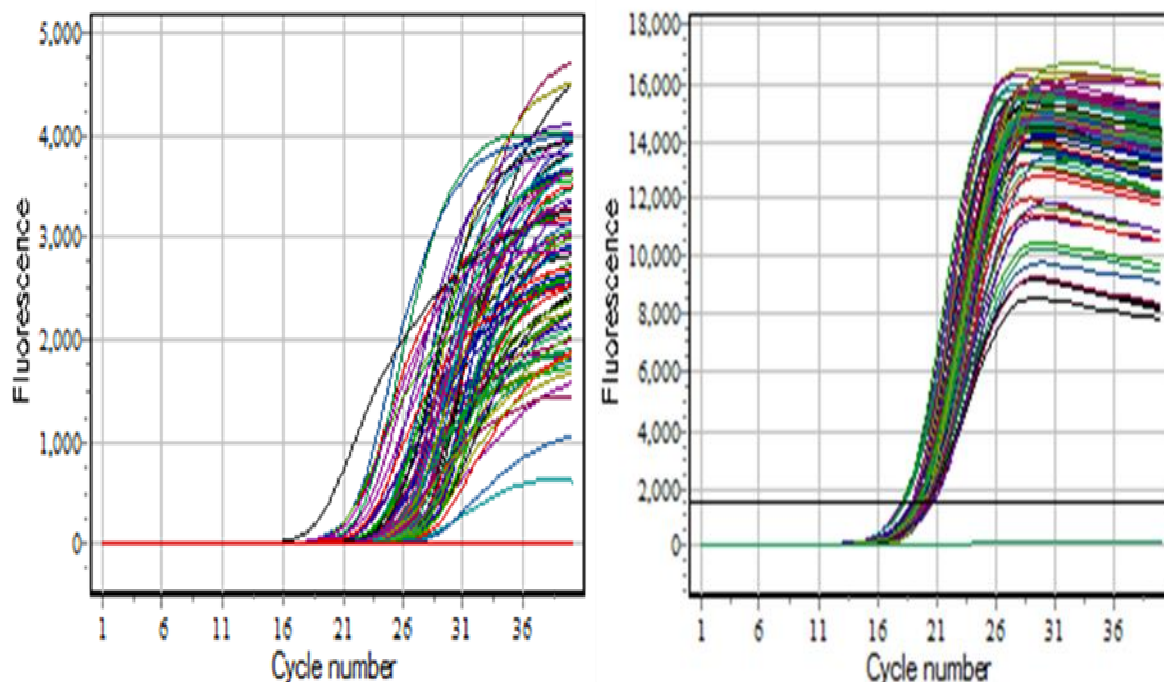

Figure (3-1): Amplification curves of APE1 gene by the Real-time – PCR

The results of gene expression revealed a significantly ($p<0.01$) higher level of APE-1 gene expression in patients than in control 3.004 ±0.15 V.S. 1.00± 0.00, As shown in Table (3-1). the q RT-PCR for APE1gene expression normalized to the GAPDH reference gene. Fold change data was calculated using the relative comparative method 2- (ΔΔCt). In this study, which may be the first in Iraq, the RT- PCR method was applied to evaluate the APE1 repair gene involved in the response of Iraqi breast cancer patients. The findings of this research revealed that patients had increased gene expression of the APE1 gene when compared to the control group, which is consistent with the findings reported in Saudi Arabia by Al Mutairi et al.,[13].

Table (3-1): Gene expression of APE1 gene.

Group	Ct, of APE1 gene	Ct of GAPDH	ΔCt	ΔΔCt	Folding
Patients	21.78	17.19	4.60	-1.38	3.004 ±0.15
Control	23.02	17.06	5.96	-0.022	1.00 ±0.00
P-value	-	-	-	-	0.0001 **
** Highly Significant (P≤0.01).					

Conclusion and Recommendations

The following conclusions were observed from the results of this study:

1. Gene expression in the patient group was found to be significantly higher than in the control group.
2. Detection of single nucleotide polymorphisms in APE1 gene in breast cancer women.
3. Estimation of the gene expression of other genes in the breast cancer patient.

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