



ORIGINAL ARTICLE

Study the expression of estrogen receptor alpha in women with breast cancer

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ABSTRACT

Binding of estrogen to ER α induced tumor growth in most ER α -positive breast cancers. The aim of this study was to determine the expression of ER α in women with breast cancer. In this study cancerous and non-cancerous paraffin-embedded tissues were collected. Total RNA was extracted. cDNA was synthesized by reverse transcription reaction. Gene expression was measured by using Relative real time PCR reaction. Estrogen receptor alpha is expressed in both cancerous and non-cancerous breast tissues but its expression in cancerous tissues is more than non-cancerous tissues (3.5 times). Statistical analysis showed that there is significant correlation between estrogen receptor alpha expression and Stage of disease ($P=0.01$). It also showed that there is significant correlation between estrogen receptor alpha expression and patient's age ($P=0.05$). Determination of estrogen receptor alpha expression can offer valuable prognostic information for clinical management of patients with breast cancer.

Keywords: Breast Cancer, Estrogen Receptor Alpha, Real Time PCR

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INTRODUCTION

Breast cancer is the most common malignancy among women worldwide and the second cause of cancer mortality [1]. Breast cancer risk factors such as early age at menarche, late age at menopause, postmenopausal hormone therapy, and high body mass index are effective in breast cancer by increasing the exposure of the breast tissue to estrogens and other ovarian hormones [2]. There is strong dissociation between estrogen receptor expression and proliferation in the human mammary epithelium, suggesting that the ovarian hormones control proliferation and development of the mammary gland indirectly via the correct secretion of paracrine growth factors. So correct secretion of ovarian steroids may be one way for reduction of the sensitivity of the mammary epithelium to the effects of the ovarian steroids and ensuring that proliferative activity occurs only when it is needed (during puberty or pregnancy) [3]. Increased ER α expression and loss of the inverse relationship between steroid receptor expression and proliferation occurs during early stages of breast cancer, suggesting that dysregulation of ER α expression is an important step in the tumorigenesis process. In fact, enhanced ER α expression would sensitize the premalignant epithelium to the proliferative effects of their natural ligands, but it remains to be determined whether estrogen continues to drive proliferation by the indirect mechanisms like what is the normal epithelium or whether an alternative [3]. Estrogen receptor alpha (ER α) are expressed in almost 65% of breast cancer cases. Binding of estrogen to ER α induces tumor growth in most ER α -positive breast cancer cells [4]. Estrogen receptor alpha can also inhibit apoptosis of breast cancer cells by dysregulation of Bcl-2 expression [5] so enhancing tumor growth.

The measurement of estrogen receptor (ER) concentrations in breast cancer tissues is an accepted method of predicting the response of a tumor to hormone therapy. Response to hormone therapy correlates with receptor positivity. So the richer tumours in estrogen receptors, are better in the prognosis for the patient [6].

Hormonal therapy was first used 100 years ago [7] and is based on blocking the activity of estrogens and their receptors [8]. Thus, measurement of the expression of estrogen receptor is an important step for

choosing the right treatment. in this study real time pcr method was used to measure the expression of estrogen receptor alpha gene.

MATERIALS AND METHODS

20cancerous and10 non-cancerous paraffin-embedded tissues were collected. RNA extraction was carried out as follows: A thin slices were prepared from paraffin-embedded tissue and were placed in a sterile micro tube. 1000 ml of xylose (MERCK,Germany) was added to the tube and put it at 56 ° C for 5 min . 1000 ml of xylose (MERCK,Germany) was added to the tube and put it at 56 ° C for 5 min . tubes were Centrifuged for 5 minutes at 13000rpm and throw away the supernatant. 1000 ml of cold absolute ethanol(MERCK,Germany) were added to the tube and the tubes were inverted 10 times. Tubes were centrifuged for 5 minutes at 13000rpm and 9 ° C, and throw away the supernatant. Steps 2, 3, 4 and 5 were repeated three times. 1000 ml protease buffer (Sina clon,Iran)and 20 ml proteinase K(Sina clon,Iran) was added to tube. Tubes were vortexed then placed for 15 min at 56 ° C, and 15 min at 85 ° C. 500 microliters of RNX-PLUS (Sina clon,Iran)added to the tubes, inverted the tube for 10 times and then Vertexed for 5 seconds. Put the Tubes for 5 minutes at room temperature. 200 ml of chloroform (MERCK,Germany) added and the tubes were vortexed for 15 seconds Then put the tube on ice for 5 minutes. Tubes were centrifuged for 15 minutes at 4 ° C with 12000 rpm. Supernatant Phase was transfered to a new tube and cold isopropanol (MERCK,Germany) was added the same volume of it. the tube was inverted 10 time then put it for 1 hour at -20 ° C. 1000 micro liters of cold 70% ethanol (MERCK, Germany)was added to the tube and the tube was inverted 10 times .The tubes were centrifuged for 8 min at 7500 Rpm and The supernatant was discarded . 30 microliters DEPC(fermentas ,United states) was added to the tubes and then were kept at -20° C cDNA was synthesized by reverse transcription reaction as follows :

1Microliter Random Hexamer primer(vivantis,Malaysia), 1Microliter oligo(dt)primer (vivantis,Malaysia) and1Microliter dntp (vivantis,Malaysia)was mixed and added to each tube. 0.5 Microliter M-MULV Reverse Transcriptase (vivantis,Malaysia) and 2Microliter of it's buffer(vivantis,Malaysia) and 4.5 Microliter DEPC water (fermentas ,United states) was mixed and added to each tube .then put them for one hour at 42° C.

Primers were designed as follows (table1):

Table1- Real time PCR sequence specific primers

<i>Genes</i>	<i>Oligonucleotide</i>	<i>Sequence</i>
ESR1	Forward primer	CACCCAGGGAAGCTACTGTT
ESR1	Reverse primer	ATCTCCACCATGCCCTCTAC
GAPDH	Forward primer	CCCACACACATGCACTTACC
GAPDH	Reverse primer	TGCCTGTCCTTCCTAGCTCT

Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was used as internal control gene.

For doing Real Time PCR, 10 Microlitr SYBR Green PCR Master Mix (fermentas ,United states), 2 Microlitr Template, 0.5 Microlitr forward primer and 0.5 Microlitr reverse primer were mixed and added to each tube. Temperature program was set as follow (table2):

Table2- time and temperature program for real time PCR

<i>Temperature</i>	<i>Time</i>	<i>Cycle number</i>
95°C	10 sec	1
95°C	5 sec	50
60°C	34 sec	50

SPSS software (version 16) was used for doing Statistical analysis.

RESULTS

Quantity and quality of RNA extraction results by spectrophotometry showed high purity and lack of pollution. amplification curves showed that the samples Were amplified without any parasit(Figure1).

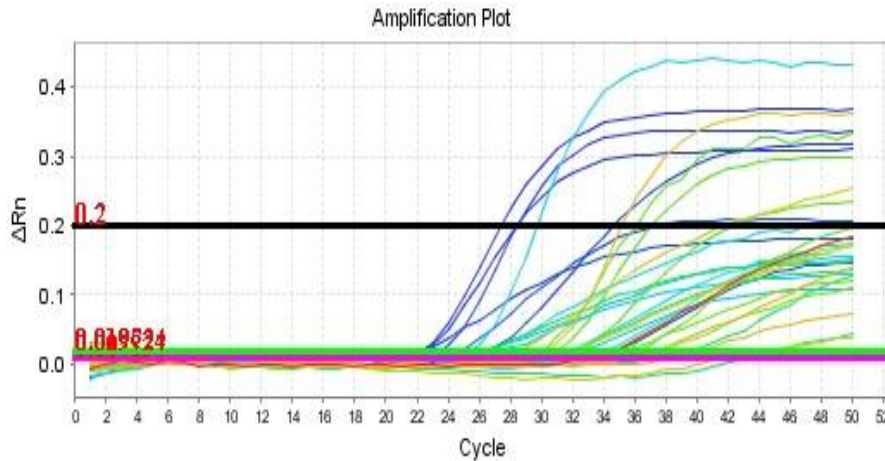


Figure1- amplification plot of ESR1

due to lack of specificity of SYBR Green color and reliability of the specific products Melting curves were plotted (Figure2).

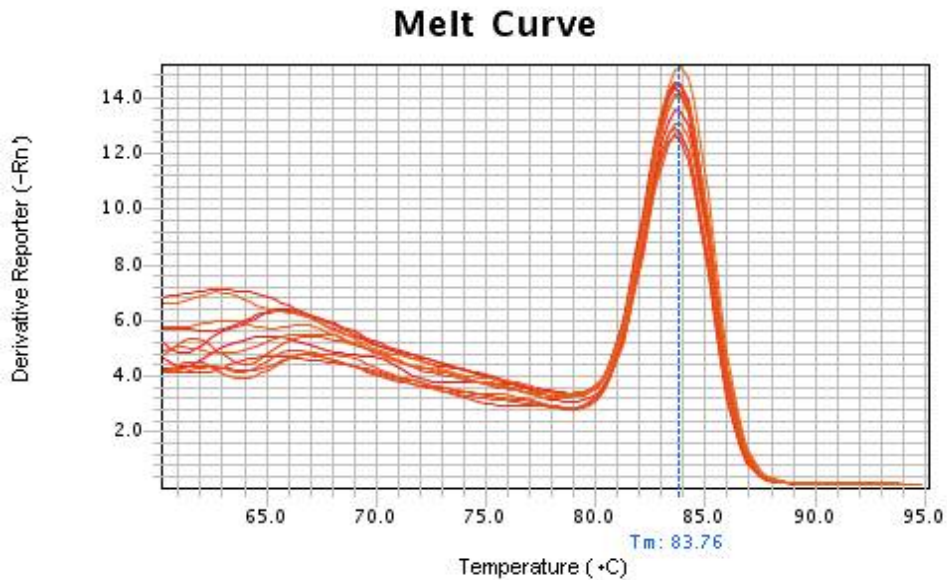


Figure2- Melting curves of ESR1

Estrogen receptor alpha Is expressed in both cancerous and non cancerous breast tissues but its expression in cancerous tissues is more than non-cancerous tissues (3.5 times).statistical analysis showed that there is significant correlation between estrogen receptor alpha expression and Stage of disease(P=0.01). It also showed that there is significant correlation between estrogen receptor alpha expression and patient’s age (P=0.05)

Table1- statistical analysis

Correlations			
	age	RQ	stage
age	1		
RQ	.324*	1	
stage	.449**	.763**	1

*. Correlation is significant at the 0.05 level

**.. Correlation is significant at the 0.01 level

DISCUSSION

Estrogen plays an important role in the initiation and progression of breast cancer.The biological effects of estrogen are mediated by its binding to estrogen receptors (ERα and ERβ)[9].ERα accepted as a

transcriptional regulator in breast cancer biology [10]. ER α functions as a ligand-dependent transcription factor that modulates gene transcription by direct recruitment to target gene [11].

Chearskul et al have studied the estrogen receptor-alpha mRNA in primary Breast Cancer. The results of their study showed that the ER mRNA and protein status were inversely related to tumor size and p53 positivity and ER protein was often positive in patients with a higher number of lymph node invasions. In their study ER protein or ER mRNA status regarding ages, menopausal status, tumor stages and histological types was not changed [12].

Suzuki et al have studied the estrogen-Related Receptor alpha in human breast carcinoma. The results of their study showed that estrogen receptor alpha immunoreactivity was detected in 55% of breast cancer tissues and was associated with mRNA level of estrogen receptor alpha. ER α immunoreactivity was shown in patients with poor prognosis. So findings suggest that ER α probably modulates the gene expression in estrogen responsive genes, and ER α immunoreactivity is a strong prognostic factor and predictive marker for tamoxifen resistance, in human breast cancer [13].

In this study we used real time pcr to assess ER α in women breast tumors. The results showed that estrogen receptor alpha gene expression in cancerous tissues was 3.5 times higher than non-cancerous. It also showed that with progression of the disease stage, the expression of estrogen receptor alpha gene increased. In conclusion, these data suggest that ER α plays different roles in gene regulation, and thus in breast cancer, and that the relative expression level of this gene should be a key determinant of cellular response to Anti-estrogen therapy.

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