RESEARCH ARTICLE

# Estrogen Receptor Alpha Gene Expression in Breast Cancer Tissues from the Iranian Population - a Pilot Study

**Arezoo Hosseini[1&], Vinod Gopalan[2&*], Mohammadreza Nassiri[1*], Kamran Ghaffarzadehgan[3], Ali Aslaminejad[1], Shahrokh Ghovvati[1], Robert A Smith[2], Alfred KY Lam[2]**

## Abstract

**Estrogen receptor alpha (ERα) is one of the major sub-types of estrogen receptors. ERα plays an important role in cellular proliferation and differentiation, chiefly in mammary tissues. In the present study we aimed to quantify of ERα mRNA and protein expression in breast tissues from the Iranian population using a real-time PCR assay. Twenty nine breast tissues including 19 adenocarcinomas and 10 normal controls were recruited from the Iranian population. mRNA extraction and cDNA synthesis were performed from these tissues using commercial kits. ERα mRNA and protein expression was quantified using real-time PCR and immunohistochemistry respectively. The results showed high expression of ERα mRNA (68%) and protein (53%) in the majority of breast cancer tissues compared to normal breast tissues (p= 0.035). Also, high ERα mRNA was associated with tumour size of breast carcinomas. In this study, we first reported the expression of ERα in Iranian patients with breast cancers and demonstrated prevalence of the expression to be similar to breast cancers noted in other populations.**

**Keywords:** Breast cancer - gene expression - ERα - relative quantitative real-time PCR - Iran

*Asian Pac J Cancer Prev,* **15 (20)**, 8789-8791

## Introduction

Previous studies suggest that during breast cancer development alterations occur in estrogen signaling pathways, mainly estrogen receptor α (ER-α) (Medina-Jaime et al., 2014). Role of estrogen receptors and its modulators in breast cancer have been widely studied in western population (Buzdar, 2013). Recently, there were Few studies have been reported recently on the genetic variance of ER-α in the Iranian population (Abbasi et al., 2009; 2012; Izadi et al., 2012). Abbasi et al. (2012) reported that single nuclear pleomorphism (SNP) s in estrogen receptor α and β have additive effects in increasing risk for developing breast cancer among Iranian breast cancer patients (Abbasi et al., 2012; Rahimzadeh et al., 2014). Also, another study reported the incidence of hypermethylation in the promoter promoter region of ER in Iranian population (Izadi et al., 2012). To the best of our knowledge, there was no study has been reported so far to determine the expression patterns of ERα mRNA or protein in breast cancer tissues due to the lack of access to human tissue samples. In this study, we aimed to measure ERα gene expression in breast cancer tissues obtained from Iranian population at both mRNA and protein. Also, some clinopathological parameters from these patients were analyzed (compared to control) in conjunction with the changes in ERα expression.

## Materials and Methods

*Selection of patients*

Formalin fixed paraffin tissues from 19 female patients diagnosed with breast carcinoma and 10 non-neoplastic (control) breast tissues were recruited from Moayyed laboratory, Mashhad, Iran. All breast cancers selected in this study were ductal adenocarcinomas and they were recruited retrospectively with no selection bias. Histopathological analysis was confirmed a hospital pathologist.

*Total RNA extraction and quantitative RT-PCR*

Total RNA was extracted using similar methods we published previously (Lam et al., 2011; Gopalan et al., 2014). Reverse transcription of the mRNA into cDNA was carried out using RevertAid™ H Minus Reverse Transcriptase kit (Fermentas, Burlington, USA). Details of the primers and amplicon size are illustrated in Table 1. A quantitative RT-PCR was used for the determination of ERα gene expression levels in the breast specimens on an ABI Prism 7300 Thermal Cycler (Applied Biosystems,

*[1]Animal Science department & Research Institute of Biotechnology, Ferdowsi University of Mashhad, [3]Department of Pathology, Razavi Hospital, Mashhad, Iran, [2]Cancer Molecular Pathology, Griffith Health Institute, Griffith University, Gold Coast, Australia [&]Equal contributors *For correspondence: v.gopalan@griffith.edu.au*

**Table 1. Features of Designed Real-Time PCR Primers (Oligonucleotide Primer Sequences) for ERα and GAPDH**

| Gene | Primer sequence | Accession number | product size (bp) | Annealing temperature |
|---|---|---|---|---|
| ERα | 5' TGGTCAGTGCCTTGTTGGATG 3'<br>5' TGTCTTGCCAGGTTGGTCAGTAAG 3' | MN_001122740 | 111 | 60°C |
| GAPDH | 5' GAAGGCTGGGGCTCATTTGA 3'<br>5' GCTGATGATCTTGAGGCTGTTGT 3' | NM_002046 | 127 | 60°C |

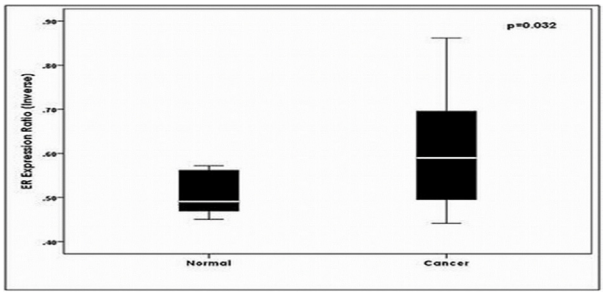


**Figure 1. ERα mRNA Expression in Normal and Cancer Breast Tissues.** The mean expression ratio (inverse ratio of ERα/GAPDH) showed high expression of ERα m RNA in breast cancer tissues compared to control samples (p=0.032)

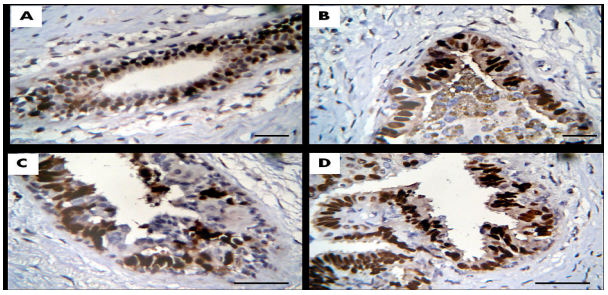


**Figure 2. ERα Protein Expression in Breast Cancer Tissues (A-D).** A strong brown staining on the nucleus of breast ductal epithelium indicates the high protein expression of ERα

**Table 2. ER Expression in Different Breast Cancer Tissues from Iranian Population**

| Type | No | Mean Age (Min-Max) | Expression Ratio (Mean±SD) | p value |
|---|---|---|---|---|
| Cancer | 19 | 50 (28-75) | 0.612 (±0.05) | 0.035 |
| Normal | 10 | 33 (19-44) | 0.505 (±0.02) | |

**Table 3. ER mRNA Expression Patterns and Correlations with Clinical in Breast Cancer Patients**

| Features | Total Number | High Expression | Low Expression | Normal Expression | p value |
|---|---|---|---|---|---|
| Age Group | | | | | |
| Age≤50 | 12 | 8 (66.7%) | 3 (25.0%) | 1 (8.3%) | 0.817 |
| Age>50 | 7 | 5 (71.4%) | 1 (14.3%) | 1 (14.3%) | |
| Size of tumour | | | | | |
| ≤20mm | 10 | 5 (50.0%) | 4 (40.0%) | 1 (10.0%) | 0.039 |
| >20mm | 9 | 8 (88.8%) | 0 (0.0%) | 1 (11.9%) | |
| Menopause status | | | | | |
| Yes | 8 | 6 (75.0%) | 1 (12.5%) | 1 (12.5%) | 0.734 |
| No | 11 | 7 (63.6%) | 3 (27.3%) | 1 (9.1%) | |

**Table 4. ER Protein Expression Patterns and Correlations with Clinical in Breast Cancer Patients**

| Features | Total Number | 0-30% Expression | 30-70% Expression | >70% Expression | p value |
|---|---|---|---|---|---|
| Age Group | | | | | |
| Age≤50 | 12 | 1 (8.3%) | 5 (41.7%) | 6 (50.0%) | 0.82 |
| Age>50 | 7 | 1 (14.3%) | 2 (28.6%) | 4 (57.1%) | |
| Size of tumour | | | | | |
| ≤20mm | 10 | 1 (10.0%) | 3 (30.0%) | 6 (60.0%) | 0.782 |
| >20mm | 9 | 1 (11.1%) | 4 (44.4%) | 4 (44.4%) | |
| Menopause status | | | | | |
| Yes | 8 | 1 (12.5%) | 2 (25.0%) | 5 (62.5%) | 0.653 |
| No | 11 | 1 (9.1%) | 5 (45.5%) | 5 (45.5%) | |

Foster city, USA) using GAPDH as a ubiquitous control.

*Immunohistochemical determination of ERα protein expression*

Immunohistochemical (IHC) staining for ERα protein was performed by the Pathology Department, Moayyed lab following routine IHC procedures. Primary monoclonal ERα antibody (ER-6F11, Novocastra, Newcastle, UK) was used at 1:50 dilution. Counterstaining was performed using 3,3-Diaminobenzidine (DAB) and Mayer's hematoxylin. Cutoff for positivity was determined at % of tumor cells staining positively for ER (i.e<1% of cells in the tumor stained was considered negative for ERα).

*PCR efficiency and data analysis*

PCR efficiency and data analysis was performed using similar methods we published previously (Gopalan et al., 2010; Lam et al., 2011; Gopalan et al., 2014). Statistical analysis was performed using the Statistical Package for Social Sciences for Windows (version 20.0, SPSS Inc., Chicago, IL, USA). Significance level of the tests was taken at p<0.05.

## Results

*High ERα mRNA expression in breast cancer tissues*

The differences in ERα mRNA expression between the breast cancer and normal tissues were significant (Table 2). The mean inverse expression ratio between ERα and GAPDH (inverse) showed high ERα m RNA levels in the breast cancer tissues compared to the normal breast tissues (mean expression ratio, 0.612 versus 0.510, p=0.032) (Figure 1). In breast cancer tissues, 68% (n=13/19) had over expression of ERα mRNA, 21% showed reduced expression (n=4/19) and 11% (n=2/19) were within the normal range.

*ERα protein expression in breast cancer tissues*

The ERα protein expression was expressed in all selected tissues with breast carcinoma. The ERα protein staining was located in the nuclei of the tumour cells (Figure 2). Similar to mRNA expression changes, ERα protein also showed higher expression in breast cancer tissues compared to control samples. High ERα protein staining (> 70% of cells showing protein staining) was noted in almost half (53%, n=10/19) of the of the selected

breast cancer tissues. ERα protein expression pattern in the remaining samples were noted as 0-30% stained cells in 11% (n=2) and 30-70% stained cells in 37% (n=5).

*Correlation analysis of ERα mRNA and protein expression with clinicopathological parameters*

All selected breast cancer tissues were clinically grouped as stage II tumours. ERα mRNA expression was noted to be high in breast cancers with bigger tumours compared to cancers with small tumours (89% over 50%, p=0.039). Also, no low expression of ERα mRNA was noted on cancers with high tumour sizes (Table 3). ERα protein expression was not correlated with any of these clinicopathological parameters.

## Discussion

Detection and quantification of estrogen receptor is a useful tool in the diagnosis and prediction of hormone therapy response in breast cancer patients. (Clark et al., 1987; Nilsson et al., 2001; Hooshmand et al., 2014). Quantification of ERα mRNA and protein expression in breast cancer tissues using real time PCR assay and immunohistochemistry has been previous reported (Bieche et al., 2001; De Cremoux et al., 2002; Chuangsuwanich et al., 2014; Wang et al., 2014). In this study, we demonstrated altered ERα mRNA and protein expression for the first time in Iranian population.

Over expression of ERα plays a major role in breast cancer pathogenesis via promoting cell growth and proliferation. This study showed increased expression of ERα mRNA and protein in breast cancer tissues compared to normal breast tissues. Also, over expression of ERα was correlated with tumour size in breast carcinoma. These results support the previous findings that ERα over expression is a common event in breast cancer population (Holst et al., 2007). Also, this finding on Iranian population shows the significant use of this gene in the molecular diagnosis and screening for Iranian women. Furthermore, ERα expression changes can be useful in selecting patients for anti-estrogen therapy in Iranian population as similar to the breast cancer management plans in western countries.

In conclusion, we have identified changes in the expression of ERα in breast cancer and normal tissues from Iranian population. In breast adenocarcinomas, ERα over expression was often noted at both mRNA and protein level. The difference in expression of ERα between normal and cancerous tissue suggests that ERα expression may be a useful surrogate molecular marker in breast adenocarcinoma. Also, these results show that ERα gene can be used as biomarker for screening and diagnosis of breast cancer patients in Iran. Further studies into this gene should be performed to identify its role in cancer development in different population.

## Acknowledgements

The authors extend special thanks to Mr. Doosti for help us to perform this work. Meanwhile, we would like special thanks from Excellent Center in Animal Science of Ferdowsi University of Mashhad, Iran and Biotechnology Institute of Ferdowsi University of Mashhad for kindly providing the funding for this research. We thank the Mouyed laboratory of Mashhad Iran for their assistance in specimen collection. This work was supported by a grant of Agricultural Faculty of Ferdowsi University of Mashhad, Iran.

## References

Abbasi S, Nouri M, Azimi C (2012). Estrogen receptor genes variations and breast cancer risk in Iran. *Int J Clin Exp Med*, **5**, 332-41.

Bieche I, Parfait B, Laurendeau I, et al (2001). Quantification of estrogen receptor a and b expression in sporadic breast Cancer. *Oncogene*, **20**, 8109-15.

Buzdar AU (2013). Meta-analysis: Selective estrogen-receptor modulators reduce breast cancer incidence. *Ann Intern Med*, **159**, 9.

Clark GM, Sledge GW, Osborne CK, et al (1987). Survival from first recurrence: relative importance of prognostic factors in 1,015 breast cancer patients. *J Clin Oncol*, **5**, 55-61.

Chuangsuwanich T, Pongpruttipan T, O-Charoenrat P, et al (2014). Clinicopathologic features of breast carcinomas classified by biomarkers and correlation with microvessel density and VEGF expression: a study from Thailand. *Asian Pac J Cancer Prev*, **15**, 1187-92.

De Cremoux P, Tran-Perennou C, Elie C, et al (2002). Quantitation of estradiol receptors alpha and beta and progesterone receptors in human breast tumors by real-time reverse transcription-polymerase chain reaction correlation with protein assays. *Bichem Pharmacol*, **64**, 507-15.

Gopalan V, Pillai S, Ebrahimi F, et al (2014). Regulation of microRNA-1288 in colorectal cancer: Altered expression and its clinicopathological significance. *Mol Carcinog*, **53**, 36-44.

Gopalan V, Smith RA, Nassiri MR, et al (2010). GAEC1 and colorectal cancer: a study of the relationships between a novel oncogene and clinicopathologic features. *Hum Pathol*, **41**, 1009-15.

Holst F, Stahl PR, Ruiz C, et al (2007). Estrogen receptor alpha (ESR1) gene amplification is frequent in breast cancer. *Nat Genet*, **39**, 655-60.

Hooshmand S, Ghaderi A, Yusoff K, et al (2014). Differentially expressed proteins in ER+ MCF7 and ER- MDA- MB-231 human breast cancer cells by RhoGDI-α silencing and overexpression. *Asian Pac J Cancer Prev*, **15**, 3311-7

Izadi P, Noruzinia M, Karimipoor M, et al (2012). Promoter hypermethylation of estrogen receptor alpha gene is correlated to estrogen receptor negativity in Iranian patients with sporadic breast cancer. *Cell J*, **14**, 102-9.

Lam AK, Gopalan V, Nassiri MR, et al (2011). Altered JS-2 expression in colorectal cancers and its clinical pathological relevance. *Mol Oncol*, **5**, 475-81.

Medina-Jaime AD, Reyes-Vargas F, Martinez-Gaytan V, et al (2014). ESR1 and PGR gene promoter methylation and correlations with estrogen and progesterone receptors in ductal and lobular breast cancer. *Asian Pac J Cancer Prev*, **15**, 3041-4.

Mousavi SM, Mohagheghi MA, Mousavi-Jerrahi A, et al (2008). Outcome of breast cancer in Iran: a study of Tehran Cancer Registry data. *Asian Pac J Cancer Prev*, **9**, 275-8.

Nilsson S, Makela S, Treuter E, et al (2001). Mechanisms of estrogen action. *Physiol Rev*, **81**, 1535-65.

Rahimzadeh M, Baghestani AR, Gohari MR, Pourhoseingholi MA (2014). Estimation of the cure rate in Iranian breast cancer patients. *Asian Pac J Cancer Prev*, **15**, 4839-42.

Wang LW, Yang GF, Chen JM, et al (2014). A clinical database of breast cancer patients reveals distinctive clinico-pathological characteristics: a study from central China. *Asian Pac J Cancer Prev*, **15**, 1621-6.