**Cukurova Medical Journal**
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ DERGİSİ

Cukurova Med J 2017;42(2):205-209
DOI: 10.17826/cutf.322799

**ARAŞTIRMA / RESEARCH**

# Association of P53 gene expression alteration among breast cancer patients in Erbil province

## Erbil'de meme kanseri hastalarında P53 geni ekspresyonundaki değişikliklerin ilişkisi

Rozhgar A. Khailany[1,2], Mustafa S. Al-Attar[3], Amer A. Hasan[4]

[1]University of Salahaddin, College of Science, Department of Biology, [2]Scientific Research Center, [3]Department of Environmental Science, Erbil, Iraq
[4]University of Sulaymani Polytechnique, College of Technical Health, Department of Medical Analysis, Sulaymani, Iraq

*Cukurova Medical Journal 2017;42(2):205-209*

**Abstract**

**Purpose:** Breast cancer is the most lethal disease and the leading cause of cancer death among women in worldwide. P53, a tumor-suppressor gene, is best known to be associated with human cancers and more than 50% of human cancers contain P53 alteration, which is guardian of the genome. In the current study, we aimed to evaluate mRNA expression level of P53 gene among breast cancer patients in Erbil province.

**Material and Methods:** Thirty four pairs breast cancer tissues and their corresponding non-cancerous breast tissues that were grouped according to the types of breast cancer and clinical features of patients were examined by semi- quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) technique.

**Results:** Expression quantity of P53 gene in breast cancer samples was significantly increased (Up-regulated) according to expression quantity of normal samples.

**Conclusion:** The over-expression of p53 gene might be a potential molecular genetics marker for breast cancer diagnosis in women; further analysis are mandatory to a better understand and confirm our preliminary findings.

**Key words:** Breast cancer, P53, expression analysis, semi-quantitative RT-PCR.

**Öz**

**Amaç:** Meme kanseri, dünyadaki kadınlarda ölümcül en büyük hastalık ve kanser ölümünün önde gelen nedenidir. P53, tümör baskılayıcı bir gen, insan kanserleri ile ilişkili olduğu bilinen ve insan kanserlerinin% 50'sinden fazlası,esas görevi genomun koruyucusu olan P53 geninin alterasyonunu içerir. Bu çalışmada, Erbil ilindeki meme kanseri hastalarında P53 geninin mRNA ekspresyon düzeyini değerlendirmeyi amaçladık.

**Gereç ve Yöntem**: Otuz dört hastaya ait meme kanseri dokusu ve yine bu hastalara ait kanserli olmayan göğüs dokuları meme kanseri tiplerine ve hastaların klinik özelliklerine göre gruplandırıldı ve yarı kantitatif ters transkriptaz polimeraz zincir reaksiyonu (qRT-PCR) tekniği ile incelendi.

**Bulgular**: Normal örneklerin ekspresyon miktarına göre meme kanseri örneklerinde P53 geninin ekspresyon miktarı önemli ölçüde arttmıştır.(upregüle).

**Sonuç**: P53 geninin aşırı ekspresyonu kadınlarda meme kanseri tanısı için potansiyel bir moleküler genetik belirteç olabilir; ön bulgularımızı daha iyi anlamak ve doğrulamak için daha fazla analiz yapılması zorunludur.

**Anahtar kelimeler**: Meme kanseri, P53, ekspresyon analizi, yarı kantitatif RT-PCR.

## INTRODUCTION

Breast cancer is the most widely recognized malignancy in women in the world[1]. It involves 22.9% on invasive cancers in women and 16% of all female cancers[2]. The frequency of breast malignancy differs significantly around the globe; it is most reduced in less developed countries and most prominent in the more developed countries[3]. Regardless of the high frequency rates, in Western countries, 89% of women determined with breast cancer are still alive 5 years after their diagnosis, which is due to recognition and treatment[3].

Breast cancer can be separated into various sorts in view of the way the tumor cells look under the microscope[4]. Most breast cancers are carcinomas, a

Yazışma Adresi/Address for Correspondence: Dr. Rozhgar A. Khailany, Salahaddin University, Science College, Department of Biology. Erbil- Iraq, E-mail: rozhgarbio@yahoo.com
Geliş tarihi/Received: 20.08.2016 Kabul tarihi/Accepted: 23.09.2016

kind of growth that begins in the cells (epithelial cells) and the second sorts are adenocarcinoma, which is carcinoma that begins in glandular tissue[4]. Analysis at the molecular level will target the basic mechanisms associated with disease development and controlling or destroying these targets are the objective of practitioners and research scientists[5].

Womens with a family history of breast tumor, have roughly two folds the danger of creating breast harms contrasted with womens without such a history of breast cancer[6]. Larger part of autosomal dominant inheritance to breast cancer connected to two genes, namely, *BRCA1* and *BRCA2*, they are tumor suppressor genes[5,6]. They help to maintain DNA stability, involves in repairing damaged DNA and destruction of cells if DNA repairment is not fruitful, and eventually results from controlled cell growth and prevention of cancer development[5,6]. Other genes are also involved in breast tumor risk[6].

Womens with the uncommon Li-Fraumeni syndrome greatly increase susceptibility to cancer, have a high danger of early breast tumor and different malignancies, that is caused by mutation in the *P53* gene[6]. The *p53* is the most as often as possible mutated gene in human malignancies and more than half of human tumors contain *p53* defects, which is guardian of the genome[7,8]. Its most pivotal ordinary capacity is liable to direct cell cycle arrest at the G1 or G2 phase of the cell cycle after specific sorts of DNA harm and to stimulate apoptosis when the impairment is too severe[9,10]. Human *P53* gene, comprises of 11 exons, which is codes a protein with 393 amino acids[11]. A few earlier studies have showed gene and protein expression of p53 via reverse transcriptase- PCR and immunohistochemistry techniques in various tumors[10,11]. In the present study we aimed to assess the conceivable relationship between mRNA expression level of *P53* and breast tumor by checking semi-quantitative RT-PCR technique, *P53* gene alteration to the risk of breast cancer.

## MATERIAL AND METHODS

### Patients

Normal and cancerous breast tissue specimens were obtained from a total 34 patients (34 controls and 34 tumors). The samples were collected from the Rizgary hospital in Erbil, north of Iraq. Twenty breast carcinoma (41-57 years) and ten breast adenocarcinoma (39-55 years) samples were enrolled in this study. Tissue biopsies were taken from the tumor and tumor free, the obtained tissues were placed into liquid nitrogen and kept at -80˚C until RNA extraction. The study was approved by the local ethics committee (approval number: 3/ 1/ 1011) and was conducted in accordance with the guidelines of the declaration of Helsinki.

### RNA extraction, cDNA synthesis and semi-qRT-PCR

RNA samples from breast biopsy tissue were gotten utilizing the extraction kit (Qiagen, Hilden, Germany) as indicated by the manufacture's direction. Quantification and qualification of total RNA concentration was performed utilizing NanoDrop (ND- 1000, USA). In this study, Complementary DNA (cDNA) was synthesized using the protoScript First Strand cDNA Synthesis Kit (Catalog no:E6300S NEB, England). The work area was cleaned by 70% (v/v) ethanol and filter tips were used in all steps. The cDNA was amplified by semi-quantitative RT-PCR and employed the expression primers[10] (Table 1). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene was used as a housekeeping gene for the normalization of *P53* gene expression data[12]. PCR reaction and condition were performed using MJ Research, AB Applied Biosystem thermal cycler. Fifty micro liter reaction mixture was prepared in PCR tubes containing 2.5 µL cDNA template, 25 µL OnePCR™ master mix (GeneDirex, Korea), 1 µL forward primer, 1 µL reverse primer and 20.5 µL ddH2O. The cycling conditions comprised of initial denaturation at 95˚C for 5 min, 35 cycles of denaturation at 95˚C for 45 sec, annealing temperatures in Table 1 for 30 sec and extension at 72˚C for 45 sec, and final extension at 72˚C for 4 min.

### Agarose gel and expression discrimination

Expression amounts were evaluated utilizing agarose gel electrophoresis (2%) in the presence of ethidium bromid. The image of agarose gel was captured and quantitated mRNA expression level by imageJ software program (version 1.46r, downloaded from http://imagej.nih.gov/ij)[13].

**Table 1. Sequence, nucleotide location, PCR product size and annealing temperature of utilized primers.**

| Primer | Sequence | Nucleotide location | PCR product (bp) | Annealing temperature (˚C) |
|---|---|---|---|---|
| P53 gene exp. | | | | |
| Forward | 5'-ACA CGC TTC CCT GGA TTG G-3' | 168-186 | 466 | 58 |
| Reverse | 5'-GGT CTT GGC CAG TTG GCA A-3' | 616-634 | | |
| GAPDH gene | | | | |
| Forward | 5'-GGTCCACCACCCTGTTGCTGT-3' | Random | 456 | 59.4 |
| Reverse | 5'-AGACCACAGTCGATGCCATCAC-3' | region | | |

### Statistical analysis

The mRNA expression level of P53 gene in breast tumor was compared with normal adjacent tissue utilizing T-test, significance was assumed for values $p \leq 0.05$. The statistical tests were made by utilizing SPSS programming (V.16).

## RESULTS

In this study, P53 gene was amplified and separated by agarose gel electrophoresis and normalized with GAPDH. Figure 1. show expression alteration of P53 and GAPDH genes. The *P53* expression level of mRNA of 34 pair samples was obtained from normal controls and tumors. Different expression level of each patient was observed, the comparison between normal controls and tumors is indicated in Figure 2. The mRNA expression level of 30 tumors according to normal controls was increased (Over-expressed).

The expression level of *P53* gene was obtained from 34 pairs; quantity of mRNA expression of *P53* tumor samples were increased according to expression level of normal control samples, p=0,0001 and statistically it is significant counted on (T- test; $p < 0,05$). The mRNA expression levels for both normal controls and tumors are shown in Figure 3.

## DISCUSSION

P53 assumes a key part in interceding cell response to different stresses, primarily by inducing or repressing various genes involved in cell cycle to arrest, senescence, DNA repair, apoptosis and angiogenesis[14]. We intended to determine possible relationship of mRNA expression level and P53 gene in breast cancer patients.

Numerous sorts of stresses might be encountered during tumour development15. The p53 function is often changed in malignancy[15]. It has been recommended that p53 could have advanced in higher organisms specifically to prevent tumour development[15]. It is trusted that this particular activity is applied fundamentally through the activating of apoptosis[15,16.] To be sure, loss of p53 action disturbs apoptosis and quickens the presence of tumors in transgenic mice[16].

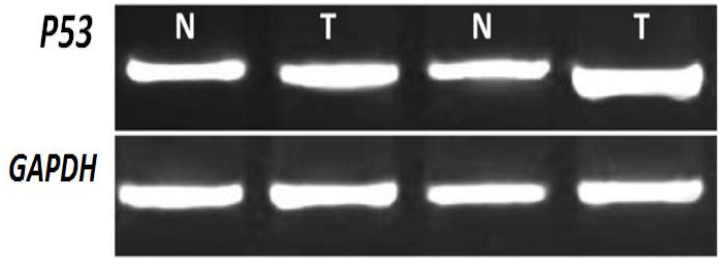


**Figure 1. The result of mRNA expression of *P53* by 2% agarose gel electrophoresis and staining by ethidium bromide. The mRNA expression level of *P53* and GAPDH genes in normal and tumor of breast cancer.**
N: normal control and T: Tumor.

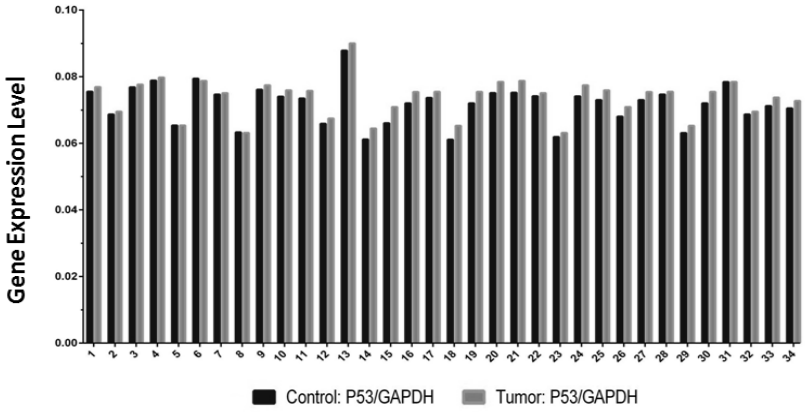


**Figure 2. The mRNA expression level of each normal control and tumor according to *P53/GAPDH*. The mRNA expression level of 30 tumors was increased.**

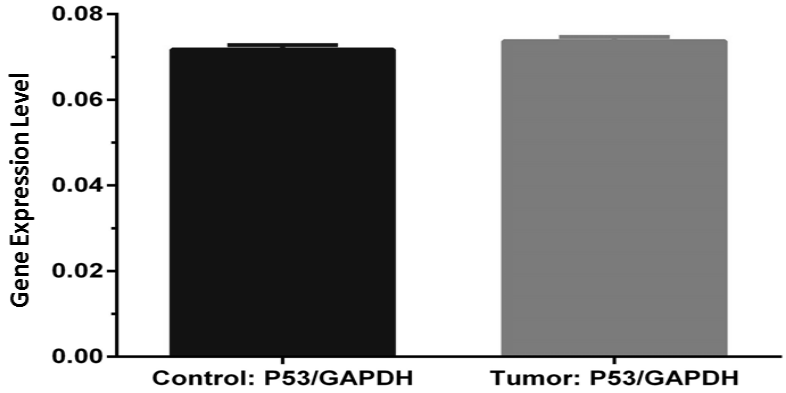


**Figure 3. Statistical results of the mRNA expression level of *P53/GAPDH* gene in both normal and tumor samples.**

Expression examination investigations of gene have issued from numerous new experiences in cancer biology and expression examination of mRNA is ending up being an extremely valuable tool for malignancy detection, cancer classification and disease resultant prediction[17]. In this study, the mRNA expression level of P53 gene was significantly increased(Up-managed) as indicated in Figure 2 and 3. Similarly, Pavel et al. (2009) reported the over-expression of P53 expression in breast cancer[18].

Posttranslational modification is a noteworthy mechanism controlling protein capacity. P53 might be phosphorylated, acetylated, cis/trans isomerized, methylated, ubiquitinated, neddylated, sumoylated, glycosylated at multiple sites, reflecting its biological significance[14]. This multisite changing, which displays a cell and tissue specificity and relies on upon the position in the cell cycle, is a complex regulatory program that fluctuates in response to cellular signalling triggered by proliferation, DNA damage, and senescence[14].

Recently, various microRNAs (miRNAs) have been observed to be involved in the p53 signaling pathway and breast carcinogenesis[19]. Certain miRNAs indirectly affected p53 signaling through regulating genes associated with p53[19]. For example, oncomiRs miR 221/222 promoted proliferation in breast cancer by repressing p53 upregulated modulator of apoptosis expression[19]. MiR 21 antagonizes the p53 pathway in breast cancer by inhibiting the expression of p53 regulated genes[19].

Another class of miRNAs directly targeted the mRNA of p53 and adversely regulate p53 expression, such as miR 125b, miR 375 and miR 504[19].

In conclusion, we observed a significant relationship between the presence of overexpression of p53 gene evaluated by semi-quantitative RT-PCR. Increased mRNA expression of P53 gene might be a risk factor for breast cancer development. In order to understand the investigation between breast cancer and molecular biomarkers; further investigation is important.

## Acknowledgements

We would like to thank to Dr. Hewa Yaseen, director of scientific research center in Salahaddin University for his permission of lab working and Dr. Mudhir Sabir Shekha for his help in statistical evaluation.

## REFERENCES

1. Bunz F. Principle of Cancer Genetics. Maryland, Springer Netherland, 2008
2. Meshram II, Hiwarkar PA, Kulkarni PN. Reproductive risk factors for breast cancer: a case control study. Online Journal of Health and Allied Sciences. 2009;8:5-10.
3. Parkin DM, Pisani P, Ferlay J. Global cancer statistics. CA Cancer J Clin. 1999;49:33-64.
4. Gerdes A, Cruger G, Thomassen M, Kruse A. Evaluation of two different models to predict *BRCA1* and *BRCA2* mutations in a cohort of Danish hereditary breast and/or ovarian cancer families. Clin Genet. 2006;69:171-8.
5. Rozhgar AK, Ari Q.N, Nadhum JI. PCR-based quantification of exon 21 in BRCA2 gene in Breast cancer patients in Erbil province. ZANCO Journal of Pure and Applied Sciences. 2016;28:94-7.
6. Morgan J, Gladson JE, Rau KS. Position paper of the American Council on Science and Health on risk factors for breast cancer: established, speculated, and unsupported. Breast J. 1998;4:177–97.
7. George P. P53 How crucial is its role in cancer?. Int J Curr Pharm Res. 2011;3:19- 25.
8. Cassidy J, Bisset D, Spence RA, Payne A. Oncology. 2nd ed. New York, Oxford University Press, 2006.
9. Liu Y, Bodmer W. Analysis of P53 mutations and their expression in 56 colorectal cancer cell lines. Proc Natl Acad Sci U S A. 2006;103:976-81.
10. Ismaiel NJ, Mohammed RA, Hidayat HJ. Gene expression of P53 and adipoq as diagnostic markers for colorectal cancer. Cukurova Med J. 2016;41:217-23.
11. Nejad A, Yaghoobi M. Mutation Analysis of TP53 Tumor suppressor gene in colorectal cancer in patients from Iran (Kerman Province). Iran J Basic Med Sci. 2012;15:683-90.
12. Ismaiel NJ, Mohammed RA, Hidayat HJ. Molecular genetics of renal cell carcinoma: polybromo 1 and set domain containing 2 genes. Cukurova Med J. 2016;41:105-11.
13. Khailany RA, Igci M, Bayraktar E, Erturhan S, Karakok M, Arslan A. VHL, PBRM1 and SETD2 genes in kidney cancer: A molecular investigation. International Journal of Medical, Health, Biomedical and Pharmaceutical Engineering. 2015;9:389-92.
14. Marc L, Robert-Alain T and Guy L. p53 and breast cancer, an update. Endocr Relat Cancer. 2006;13:293–325.
15. Vousden KH, Lu X. Live or let die: the cell's response to p53. Nat Rev Cancer. 2002;2:594-604.
16. Attardi LD, Jacks T. The role of p53 in tumor suppression: lessons from mouse models. Cell Mol Life Sci. 1999;55:48-63.
17. Chungyeul KM, Yusuke TM, Soonmyung PM. Gene-expression-based prognostic and predictive markers for breast cancer - A primer for practicing pathologists. Arch Pathol Lab Med. 2009;133:855-9.
18. Pavel RJ, Marilie DG, Yu-Jing ZA, Mary BT, Hanina HE, Lorenzo MF et al. Mutations in p53, p53 protein overexpression and breast cancer survival. J Cell Mol Med. 2009;13:3847-57.
19. Enxiang Z, Na H, Min S, Baiping W, Jianlin Z. Systematic analysis of the p53-related microRNAs in breast cancer revealing their essential roles in the cell cycle. Oncol Lett. 2015;10:3488-94.