The Effect of PTEN Gene Expression on Thyroid and Breast Cancer in Iraqi patients

Rana Shamil Noori[1]*, Zahraa Abdul Munaam Abdul Hadi[1], Israa Hussein Hamzah[2]

[1] Department of Applied Science, University of Technology Baghdad. Iraq
[2] College of Sciences, Mustansiriyah University, Baghdad. Iraq
Email ranashamel87@gmail.com

Abstract

This study was conducted to cover the effects associated with the PTEN gene and its relationship to thyroid cancer and breast cancer in familial mutations. The total number of samples used was 150 samples distributed as follows, the first group was 100 samples between the ages of 18-85 years, divided into 50 women with thyroid cancer, and another 50 samples from women with breast cancer, and the second group 50 samples, women The healthy, unaffected subjects aged between 18-85 years. Samples were taken in Baghdad / Al-Amili, Iraqi National Hospital. From hospital archives and case records, we learned how to treat cancer and what its symptoms are. 2 ml of blood sample was drawn from each person and PTEN gene expression and its relationship to the total were measured. The value of 2 carats for the health kit is 3.146E-07. For the thyroid group, it was 3.372E-07. The breast cancer group was 3.372E-07. The calculated ratio of fold gene expression was 1.00 for the healthy groups and 1.07 for the thyroid cancer group for the breast cancer group. In conclusion, the high toxicity of the PTEN gene was demonstrated in women carrying the gene and detected with breast cancer by 6 times and in women with thyroid cancer by 3 times, while healthy women were free of the gene.

Keywords: **Gene expression, PTEN, Thyroid cancer, Breast cancer**

1. Introduction

Thyroid cancer, the most frequent endocrine system tumor, arises from follicular thyrocytes parafollicular C-cells of the thyroid gland and represents a pattern of malignant change from benign adenomas and highly diversified squamous cell carcinoma to moderately differentiated carcinomas [1]. It is most common in people over the age of 60 and in people in their 30s. Females are 2 to 3 times more likely than men to acquire it. Thyroid cancer is generally treatable and can be fully healed in certain circumstances, but occasionally it may come back after treatment [6]. It can start from the tissues of the gland or another location after spreading and reaching it, as the gland is located in the front of the neck and under the larynx [2]. Breast cancer is considered the second most common cancer worldwide and the most frequently diagnosed life-threatening cancer in women [3]. Abnormal growth and division of breast cells and tumor or lump development are referred to as breast cancer. Typically, these cells inhibit the ducts that are the milk carrier of the nipple tubes or the gland that contain the milk known as the lobules [4,5] . Genetic predisposition to breast cancer. Associated with germline mutations in PTEN gene. Combined, these two Genes appear to be responsible for the majority of inherited breast cancer cases [12]. So far, mutations in either genos have not been able to explain a large proportion (about 20–40%) of the apparent familial cases [13]. Three known germline mutations in PTEN and its link to breast and thyroid cancer are most common in Ashkenazi Jews and cause about 40–50% of familial cases of breast and thyroid cancer [14]. Also, a full analysis of mutations in high-risk genes in Iraqi women found only a few more mutations [15, 16]. So, it seems likely that a tendency to get breast cancer could be caused by changes in other genes. potential candidate gene PTEN. In this study, we looked for germline mutations in PTEN in two groups of Iraqi patients: People who have been told they have PTEN phenotype.And women who have a clear genetic predisposition to breast cancer, and some of them have prominent features that are mentioned, according to statistics taken from the Iraqi Hospital for Cancer Tumors.

Materials and Methods

This study was taken place during the period from October 2021 to May 2022. All the study experiments were performed at the University of Technology and Iraq Hereditary Company (IHC).

Study Groups

The overall number of people involved in the research was 150 people, with the respective study groups: - GROUP1.100 patients' blood samples divided into 50 patient blood samples of Iraqi men and women diagnosed with thyroid cancer and 50 patient's blood samples of Iraqi women diagnosed with breast cancer aged between 40 and 50. (18-85 years). The samples were taken in Baghdad/Al-Amel , Iraq's National Hospital for Cancer Treatment and clinical information was obtained from hospital archives and case-sheet records .

doi.org/10.31838/hiv23.01.17 Received: 18.09.22, Revised: 12.10.22, Accepted: 13.11.22

Group 2: 50 samples were collected from seemingly healthy persons of both sexes ranging in age from 21 to 85 years.

Blood Sampling

From each participant , 2 ml of whole blood must be collected from the venous blood into an EDTA containing tube ; this procedure was done under aseptic conditions.

TotaliiiRNAiExtractionifrom BloodiiiiSamples

The RNA was extracted from whole blood samples of patients and healthy controls using the TransZolUp plus RNA Kit [7].

DNA synthesis from mRNA

Total RNA was reversely transcribed to complementary DNA (cDNA) using Easy Script®OneStep gDNA Removal and cDNA Synthesis Super Mix Kit (TransGen, China) [8, 9]. The procedure was performed in a reaction volume of 20 µl, according to the manufacturer's instructions. The total RNA volume for reverse transcription was 20µl. Thermal cycle steps of cDNA reverse transcription conditions are presented in table 2. Primers used in this study and their sequences are tabulated in table 1

Table 1. Thermaliiicycleristeps			
	Step1i	Step2i	Step3i
Temperature/ °ci	25	42	85
Timeiii	10iiimin	15iiimin	5iiiseconds
	RandomiiiPrimer (N9) binding	AnchorediiiOligo(dT)18 binding	Inactivatei reverseiiii transcriptase enzymeiii

Primers used in the study are shown in (Table 2) for GAPDH and PTEN genes.

Primer	Sequence (5'→3' direction)
	PTEN qPCR expression Primer
Forward	GGACGAACTGGTGTAATGATATG
Reverse	TCTACTGTTTTTGTGAAGTACAGC
	GAPDH –housekeeping gene
Forward	TGAGAAGTATGACAACAGCC
Reverse	TCCTTCCACGATACCAAAG

Real-Time PCR (qRT–PCR) The qRT-PCR test was performed using the Stratagene Real-time PCR System (Analytik JenaTechnologies) with qPCR software [10]. The gene expression levels and fold changes were quantified through the measurement of the threshold cycle (Ct) using the 2xqPCR Master Mix Kits (Trans Gen, China) [11, 12]. The components are presented in table 3. Every reaction was performed twice and included a non-template control, non-amplification control, and non-primer control (NPC) as the negative controls. **The PCR Reaction Run** The cycling protocol was set up following the thermal profile described in (Table 4).

Housekeeping Gene Amplification The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was employed as an internal control in calculating the ΔCT value. Table 5 shows the temperature profile used in a qRTPCR process for GAPDH amplification.

RT-PCR analysis of PTEN gene expression

The expression ratio was calculated without a calibrator sample 2-ΔCt, according to the following equation:

ΔCT (test)= CT gene of interest (target, test) – CT internal control

Eventually, the expression ratio was calculated according to the following formula: 2- ΔCt = Normalized expression ratio

Table 3. Reaction Component and volumeiiifor RT PCR usediiniPTEN geneiiiii Component	
Component	**Volume (µl)**
Master mix Syper Green	10
Forward primer	1
Revers primer	1
CDNA	2
Nuclease-free water (N.F.W)	6
Total volume	20

Tableiii4. Thermaliprofileiiiof PTEN, geneiiiexpression.				
		Temperature	Time	cycle
Stage 1	Denaturation	95°C	30 sec	1
Stage 2	Denaturation	95°C	5 sec	
	Annealing	60	15 sec	40
	Extension	72°C	20 sec	
Stage 3	Dissociation	55-95	1	1

Table 5. Thermaliprofileiiiof GAPDHigeneiiexpression		
Step	Temperature/ °C	Duration/ Sec
Enzymes activation	95	30
Denature	95	5
Anneal	60	15
Extend	72	20

Results and Discussion

The Ct value of GAPDH, the housekeeping genes used in the present study, is shown in Table (6). The Ct value for GAPDH in the healthy group was a mean ± SEM (21.53± 0.15). For the thyroid cancer with a mean ± SEM (21.47 ± 0.06). In the breast cancer group, with a mean ± SEM (20.97± 0.07). A significant difference was found between these groups between the mean Ct value of GAPDH (*P*-value = 0.00).

Barber [13] used qRT-PCR to examine the expression of 1,718 genes in 72 different types of normal human tissue, using GAPDH as a reference gene. When used in clinical research, they discovered that GAPDH is a reliable technique for normalization in qRTPCR.

To improve on this, and even though there was a significant variance in the mean Ct value between groups in the current study, the variability of the total change in GAPDH expression was studied in separate study groups using the 2-Ct value and the ratio of 2-Ct of the different study groups to that of the control group, as shown in Table (7)

The 2-Ct value of the healthy group was **3.146E-07**. for the thyroid group, it was **3.372E-07.** And the breast cancer group was **3.372E-07**. The computed ratio for gene fold expression was **1.00** for the healthy groups and **1.07** for the thyroid cancer group for the breast cancer group **1.07**. These minor variations in gene fold expression between the study groups render the GAPDH gene functional control gene

Table 6. Theihousekeepingigeneiii (GAPDH) Ct values the three studied groups.			
Groups ii	CT GAPDH house-keeping gene		
	N	Mean ± SEM	Median
Thyroid Cancer	50	21.47 ± 0.06	21.36 a
Breast Cancer	50	20.97± 0.07	20.92 b
control	50	21.53± 0.15	21.10 a
Kruskal-Wallis *P*-value ii	0.00		

highly significant $P \leq 0.001$ (***)

Table (7): Comparison of GAPDH Foldiexpressionibetween study groups.				
Group	Means Ct of GAPDH	2^{-Ct}	experimental group/ Control group	The fold of gene expression
Group 2 Thyroid Ca.	21.5	3.372E-07	3.372E-07/3.146E-07	1.07
Group 2 Breast Ca.	21.5	3.372E-07	3.372E-07/3.146E-07	1.07
Group 1 control healthy	21.6	3.146E-07	3.146E-07/3.146E-07	1.00

Each quantification PCR reaction was run in triplicate for each sample. Samples from the healthy and cancer groups were used in each run. Plots of each run (i.e., amplification plots and dissociation curves) were also recorded. Figures 1, 2, 3, and 4 in the appendix present the amplification plots and dissociation curves for GAPDH and PTEN, respectively.

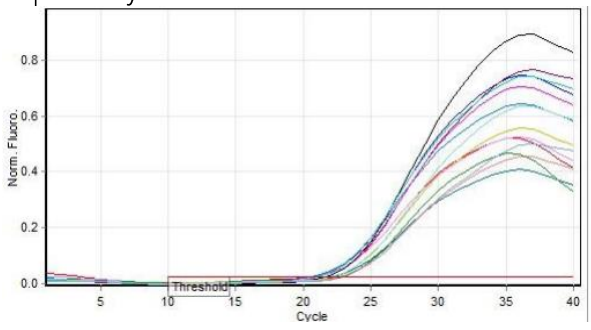

Figure 1. GAPDH amplification plots by qPCR Samples included all study groups. The photograph was taken directly from the Qiagen rotor Gene qPCR system.

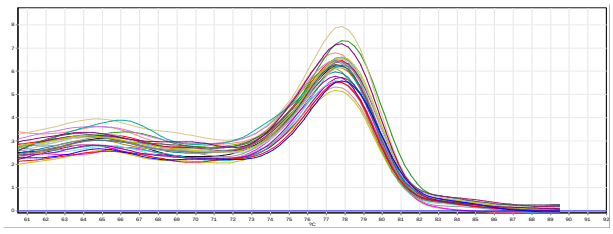

Figure 2. GAPDH dissociation curves by qPCR Samples included all study groups. The photograph was taken directly from the Qiagen Rotor gene qPCR machine.

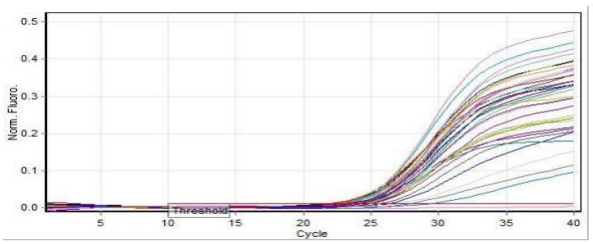

Figure (3) PTEN Amplification plots by qPCR Samples included all study groups. The photograph was taken directly from Qiagen a Rotor gene qPCR machine.

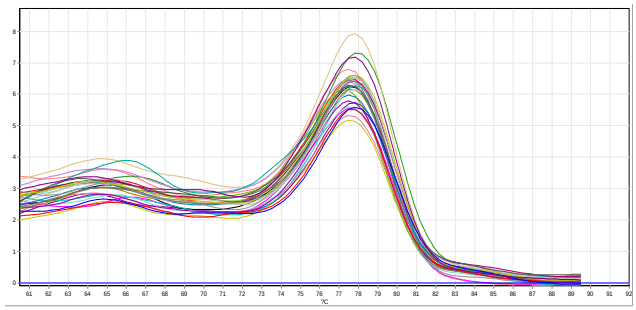

Figure (4) PTEN dissociation curves by qPCR Samples included all study groups. The photograph was taken directly from the Qiagen Rotor gene qPCR machine.

In the present study , a quantitative RT-PCR assay analyzed the mRNA expression of PTEN and compared its expression between the healthy group, thyroid cancer group, and breast cancer group. The calculation of gene expression fold change was made using relative quantification.

This depends on the normalization of Ct values calculating the ΔCt, which is the difference between the mean Ct values of the replica of PTEN cDNA amplification of every single case and that of the GAPDH.

To calculate the gene expression folds concerning the housekeeping genes, the result of 2- ΔCt of each group was measured concerning that of the control group.

The results are shown in Table (8). The fold of gene expression in the thyroid cancer group was 6 times higher than in the healthy group. The breast cancer group was 2 times higher than the healthy group. These results indicate significantly increased expression of the PTEN gene in these groups.

All study groups were divided into two subgroups, high expression when the fold gene expression change was above 1 and low expression when the fold change was lower than1.

It is well-known here that the high expression was evident in the thyroid cancer group compared to the healthy group. However, the breast cancer group also shows many high-expressing individuals. The Induction of PTEN gene expression is partly due to cancer itself.

Our results show high expression in the PTEN gene in all types of cancers under study, which doesn't agree with the mechanical work of the PTEN gene.

This result locally agreed with [14] that Tumor PTEN gene expression was significantly increased in patients living in the areas of high-risk depleted uranium exposure in comparison to patient tumors from low-risk areas. And agreed with [15] [16]. Unexpectedly , PTEN mRNA expression was significantly higher in breast carcinoma tissues compared to normal breast tissue.

And doesn't agree with [17] [18] Our results suggest that down-regulation of PTEN expression at the mRNA level plays a role in PTEN inactivation in thyroid cancer, and PTEN exerts its tumor-suppressive effection thyroid cancer through the inhibition of cell cycle progression alone or both cell cycle progression and cell death. PTEN is also recognized as a tumor suppressor, and it was observed that PTEN is inactivated or inhibited in multiple types of cancer, including thyroid carcinoma research over the past few years has shown the mechanism by which loss of PTEN function contributes to tumor development

And does not agree locally with [19]. Loss of the PTEN gene can cause overgrowth, proliferation, survival, and metabolism of tumor cells. In breast cancer, loss of the PTEN gene is associated with the occurrence of tum ors and is significantly correlated with its characteristics. And Inactivation of PTEN, and thus lack of inhibition of the AKT-dependent processes, has been associated with tumor genesis in multiple human cancers,including breast cancer [20].PTEN loss was significantly higher in breast cancer tissues than in matched normal tissues, which

suggests that PTEN might play an essental role in th e development of breast cancer. [6]

Table (8) Fold of *PTEN* expression Depending on $2^{-\Delta Ct}$ Method

Groups	Means Ct of *PTEN*	Means Ct of GAPDH	ΔCt (Means Ct of *PTEN* - Means Ct of GAPDH)	$2^{-\Delta Ct}$	experimental group/ Control group	The fold of gene expression
Group 2 Thyroid Ca.	21.807	21.500	0.307	0.808	0.808/0.117	6.881
Group 2 Breast Ca.	23.264	21.500	1.764	0.294	0.294/0.117	2.508
Group 1 control healthy	24.690	21.600	3.090	0.117	0.117/0.117	1.000

Conclusion

The aim of the study and as our results showed a high expression of PTEN gene in all types of cancers under study, periodic examination of the gene should be carried out, especially in families in which breast cancer and thyroid cancer recur.

Ethical procedures performed in this study involving human participants were following the ethical standards of the University of Technology, Baghdad

Acknowledgment

The authors would like to thank the faculty and staff members of Applied Science Department, Biotechnology division, University of Technology, Baghdad, Iraq, and Iraqi Hereditary Company (IHC), Baghdad, Iraq.

References

1. Samar Abdul Raheem Shaghati. [2015]. Detection of Parvovirus B19 by Immunohistochemistry in

Patients with Thyroid Tumors A Thesis Submitted to the Council of College of Science AL-Mustansiriyah University in Partial Fulfillment of The Requirements For The Degree of Doctorate of Philosophy in Biology/ Zoology.
2. Haugen, B. R. (2017). 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: what is new and what has changed? Cancer, 123(3), 372-381.
3. Al-Khafaji, H. M. (2017). Gene Expression Bcl-2 Gene in Cancer of Brest in Iraqi population. *Engineering and Technology Journal*, *35*(2 Part B), 119-125
4. Akram, M; Iqbal, M; Daniyal, M; and Khan, A. U. (2017). Awareness and current knowledge of breast cancer. Biological research, 50(1), 33.
5. Milella, M., Falcone, I., Conciatori, F., Cesta Incani, U., Del Curatolo, A., Inzerilli, N., Nuzzo, C. M., Vaccaro, V., Vari, S., Cognetti, F., & Ciuffreda, L. (2015). PTEN: Multiple Functions in Human Malignant Tumors. Frontiers in oncology, 5, 24.
6. Li, S., Shen, Y., Wang, M., Yang, J., Lv, M., Li, P., Chen, Z., & Yang, J. (2017). Loss of PTEN expression in breast cancer: association with clinicopathological characteristics and prognosis. Oncotarget, 8(19), 32043–32054.
7. Chi, S., Xue, J., Li, F., Zhu, C., Yu, Y., Li, H., Wang, X., Zhang, Y., Yang, J., Zhou, S., Yang, L., Ji, C., & Liu, X. (2016). Correlation of Serum Soluble Interleukin-7 Receptor and Anti-C1q Antibody in Patients with Systemic Lupus Erythematosus. Autoimmune diseases, 2016, 8252605.
8. Ghareeb, Z. A., AlKhafaji, H. A. H., & Al-Saedi, M. K. A. (2021). The Effect of LRP-1 Gene and Mir-205 on cardiovascular disease in Iraqi Patients with Familial Hypercholesterolemia. *Journal of Applied Sciences and Nanotechnology*, *1*(3), 24-31.
9. Li, S., Tian, Y., Wu, K., Ye, Y., Yu, J., Zhang, J., Liu, Q., Hu, M., Li, H., Tong, Y., Harberd, N. P., & Fu, X. (2018). I am modulating plant growth-metabolism coordination for sustainable agriculture. Nature, 560(7720), 595–600.
10. Abed SA, Al-Khafaji HM. (2021) Design of Primer and Probe To Detect SNP Rs 1892901 In Fosl-1Gene In Different Types Of Cancer In Iraqi Population. Syst Rev Pharm.;12(1):296-300.
11. Al-Saadi, B. Q. H., Al-Khafaji, H. M. A. H., & Al-Saedi, M. K. A. (2021). Primer and Probe Designing to Detect SNP rs 4073 in Interleukin-8 Gene in Iraqi Patients with Bronchial Asthma. *Journal of Applied Sciences and Nanotechnology*, *1*(3).
12. Yang T-b, Liu J, Yuan L-y, Zhang Y, Li D-q, Agnarsson I, et al. (2017) Molecular identification of spiders preying on Empoasca vitis in a tea plantation. Sci Rep.;7(1):1-10
13. Arrouchi, H., Lakhlili, W., & Ibrahimi, A. (2019). A review on PIM kinases in tumors. Bioinformation, 15(1), 40–45
14. Jumaah, A.S., Al-Haddad, H.S., Mahdi, L.H., et al. (2019). increased PTEN gene expression in patients with endometrial carcinoma from areas of high risk depleted uranium exposure. BMC Res.
15. Palimaru, I., Brügmann, A., Wium-Andersen, M. K., Nexo, E., & Sorensen, B. S. (2013). Expression of PIK3CA, PTEN mRNA and PIK3CA mutations in primary breast cancer: association with lymph node metastases. SpringerPlus, 2(1), 464.
16. Alowiri, N. H., Hanafy, S. M., Haleem, R. A., & Abdellatif, A. (2019). PIK3CA and PTEN Genes Expressions in Breast Cancer. Asian Pacific journal of cancer prevention: APJCP, 20(9), 2841–2846.
17. Liang-Ping Weng, Oliver Gimm, Jennifer B. Kum, Wendy M. Smith, Xiao-Ping Zhou, David Wynford-Thomas, Gustavo Leone, Charis Eng(2001) Transient ectopic expression of PTEN in thyroid cancer cell lines induces cell cycle arrest and cell type-dependent cell death. Human Molecular Genetics
18. Salmena L. (2016). PTEN: History of a Tumor Suppressor. Methods in molecular biology (Clifton, N.J.), 1388, 3–11.
19. Mohammed R.F. Al-Taie, Ban J. Qasim (2021), Assessment of PTEN Deletion or Signal Reduction in Breast Carcinoma Using FISH Technique in A Sample of Iraqi Female Patients, IRAQI JOURNAL OF MEDICAL SCIENCES
2021, Volume 19, Issue 2
20. Zhang, S., & Yu, D. (2010). PI(3) king apart PTEN's role in cancer. Clinical cancer research: an official journal of the American Association for Cancer Research, 16(17), 4325–4330.