Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.47750/jptcp.2023.30.08.006

Expression of Tansketolase TKTL1 in Iraqi Breast Cancer Females

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Submitted: 10 February 2023; Accepted: 17 March 2023; Published: 06 April 2023

ABSTRACT

Background: Breast cancer is the second leading cause of cancer death among women around the world.TKTL1 is major enzyme in the non oxidative reaction in the pentose phosphate pathway (PPP) pathway lead to lactate production and only increases aerobic glycolysis that contributes to carcinogenesis. The aim of this study is to investigate the TKTL1gene expression in breast cancer patients and its relationship with patients clinical characteristics. .

Methods: The study included detection of TKTL1gene expression in 50 Iraqi breast cancer patients and 25 healthy controls using qPCR technology.

Results: Increasing level of folding gene expression (FE) for the TKTL1 gene compared to the control group was found in breast cancer females. No significant differences in TKTL1 gene expression depend ages were found in this study. It was observed that the level of FE on the right side increased by an average 5.06 in 46% (23/50) compared with 4.22 fold on the left side in 54 % (27/50). Significant differences depending on the tumor site ($P \leq 0.05$) were observed in this study. An increase of FE (4.96) for the TKTL1 gene was found in Invasive Ductal Carcinomas (IDC) of breast tumors with a significant association. The results also showed a high rate of TKTL1 FE (4.65) in 78% (39/50) of patients with The second grade of tumor compared to (1.48) in the control group with high significant association ($P \leq 0.01$). A significant association between FE of TKTL1 gene expression and tumor grade ($P \leq 0.01$). The results refer to an increase of FE (6.39) for KTL1gene in 12% (6/50) and (4.90) in 42% (50/21) patients with stage I stage and II respectively The results were showed significant differences depending on breast cancer stage ($P \leq 0.01$).

Conclusions: High level of TKTL1gene expression gene was found in Iraqi females with breast cancer with significant relation depending on site, type, second grade, and stage of breast cancer on the right side. The study indicated that the expression of TKTL1gene maybe act as a biomarker for early detection of breast cancer.

Keywords: *Trasketolase, , Breast cancer, Gene expression, Iraq*

INTRODUCTION

Breast cancer is the most common type of cancer diagnosed among women worldwide, it is a heterogeneous disease at the molecular level(1). and is the second leading cause of cancer death among women around the world and in the United States, according to the American Cancer Society, 268,600 women have been diagnosed with breast cancer. In 2019 and 41,760 of them died of the disease(2). In Iraq, breast cancer has become a major public health problem and its burden increases with the increase in population size. The Iraqi Cancer Registry reveals that 25,556 new cases of cancer were registered in 2016. Breast cancer is the most common malignant tumor(3). A hallmark of cancer cells is metabolic reprogramming, whereby cancer cells tend to produce ATP via anaerobic glycolysis rather than oxidative phosphorylation in mitochondria even when oxygen is available. Increased glycolysis and the pentose phosphate pathway PPP regulate cancer cells to adapt to the high demand for large molecules for proliferation and to maintain reactive equilibrium during malignancy progression(4).

The pentose phosphate pathway(PPP) is one of the metabolic pathways that occur in living cells to produce energy and maintain cellular homeostasis(5). It is the main regulator in cellular interaction, oxidation, reduction and biosynthesis. The enzymes of this pathway have an important role in many human diseases, including cancer and diabetes(6). This pathway consists of two branches, the first is the oxidizing and the second is the non-oxidizing branch

The non oxidative part of the pentose phosphate pathway is controlled by transketolase enzyme reactions, and there are three genes in the human genome: TKT, TKTL, and TKTL2(7).

TKTL1 is a major enzyme in the non-oxidative reaction in the PPP pathway to lactate production and not only increases aerobic glycolysis that contributes to carcinogenesis. Inhibition of TKTL1 gene expression leads to a decrease in the proliferation rate and a decrease in glucose metabolism, so the high expression of TKTL1 may be act as a marker for the cancer cells proliferation(8). TKTL1 gene expression is upregulated in various types of human cancers such as head and neck, breast, colon, stomach, endometrial, and melanoma (9) . Our previous article was concluded that PTEN gene may act a promising biomarker in detection of breast cancer patients and predicting the prognostic state of those women (10) while This study was aim to investigate the TKTL1 gene expression in breast cancer patients and their relationship with clinical characteristics Because of the increased incidence of breast cancer and the lack of biomarkers for early detection of these malignant tumors.

MATERIALS AND METHODS

Subjects

This study include 50 blood samples that were collected from breast cancer patients in addition to 25 blood samples from apparently healthy individuals as a control group. Samples were collected from the Oncology Teaching Hospital of the Medical City in Baghdad, from December, 2020 to February,2021.The range of s patients age were (30-71) years with average age 51 years from different area of Baghdad and other governorates. Ethical permission to conduct the research was obtained from the Hospital and from all participants in the study. Selections of the patients and diagnosis were done under the consultant’s medical staff and pathologist committee at the Oncology Teaching Hospital of the Medical City in Baghdad. The diagnosis was made according to the clinical mammographic, histological findings, by which patients were early detected. None of the patients received chemotherapy or radiotherapy or treatment with mastectomy before blood collection. The molecular detection was carried out by using conventional quantitative polymerase chain reaction(qPCR)technique.

RNA Extraction and qPCR

Total RNA was extracted from blood samples using TRIzol Reagent (Thermo Scientific, USA). One-Step RT-PCR was used in this study. total volume of reaction was 10 µl including (5µl qPCR Master Mix, 0.25 x RT mix .0.25 MgCl2 and 0.5 µM for each (forward and reverse) primer, 2.5 µl Nuclease Free Water and 1 µl of RNA). Detection of TKT gene expression level was done by RT-qPCR technique using (Mic qPCR Cycler, Bio Molecular System, Australia).

GAPDH gene was used as housekeeping gene in this study, Table (1) refer to primers sequences TKTL1(11). GAPDH(12)

TABLE 1: The Primers sequences used for - qPCR

Primer Name	Sequences
TKTL1-F	5`-TAACACCATGACGCCTACTGC-3`
TKTL1-R	5`-CATCCTAACAAGCTTTCGCTG-3`
GAPDH-F	5`-GAAGGTGAAGGTCGGAGTC-3`
GAPDH-R	5`-GAAGATGGTGATGGGATTC-3`

The RT-qPCR was performed with the following conditions the first cycle was include (RT. enzyme activation at 37 °C for 15 min, initial denaturation 95°C for 10 min), followed by 40 cycles of 95 °C for 20 sec, 60 °C for 20 sec and 72°C for 20 sec. The glyceraldehyde phosphate dehydrogenase (GAPDH) was used (as a housekeeping gene). (Table 1) showed the primers that were used for qRT-PCR reactions, all primers were purchased from (Macrogen, Korea). The results of gene expression was analysed using Pfaffi Method (Folding = 2-ΔΔCT, ΔΔCT =ΔCT Treated - ΔCT Control, ΔCT =CT gene - CT House Keeping gene).

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test was used to significant compare between means.Chi-square test was used to significant compare between percentage(0.05 and 0.01 probability) in this study.

RESULTS AND DISCUSSION

TKTL1 Gene Expression

Previous study was concluded we believe that PTEN gene is a promising biomarker that could be useful in detection of breast cancer patients and predicting the prognostic state of those women.

The results of TKTL1 gene expression of breast cancer patients showed that folding expression (FE) level was increased four times by 4.6 compared to 1.3 the gene expression of control group samples. The CT values ranged between (-22.04-42.28) and the Δ CT values were (0.25-9.82), while the ΔΔ CT values were (13.76-0.11) in patients. Figure (1) showed sharply defined melting curves with narrow peaks for RT- qPCR products for TKTl1 gene indicating for pure and homogeneous products.

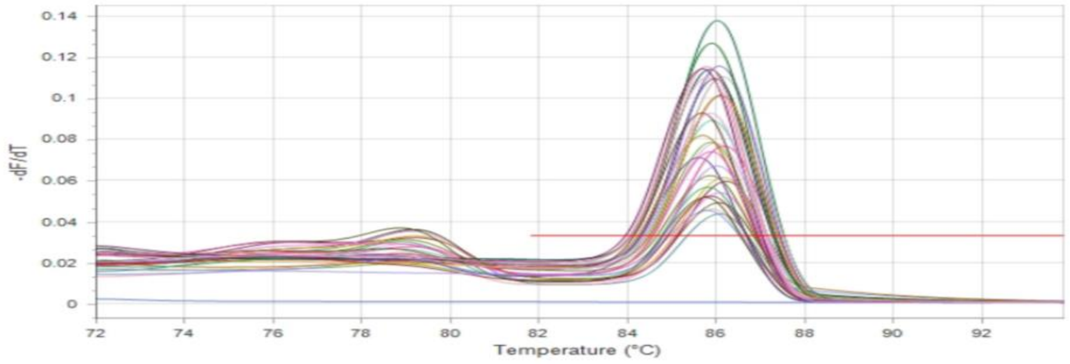

FIGURE 1: Melting curve of TKTL1 gene.

Relationship of TKTL1 Gene Expression Level with Age and Location

Table (2) indicates to the level of TKTL1 gene expression depending on age and location. Although an increasing of the TKTL1 FE gene expression (5.46) was observed in the older ages($\geq$ 50 years) in 60% (30/50) of patients compared to the 4.89 in 40% (20/50) of patients less than 50 years, but no significant differences of TKTL1 gene expression depend ages were found in this study. Ahopelto and his colleagues group (13) refer to similar results in stomach cancer patients, also our results keep with study by (14) in colorectal cancer patients. No other study investigated the level of TKTLI gene in breast cancer was found.

As for the location of the tumor, it was observed that the level of FE on the right side increased by an average of 5.06 in 46% (23/50) compared with 4.22 fold in left side in 54 % (27/50). Significant differences between the right and left sites ($P \leq 0.05$) was observed in this study. The presence results did not agree with the results study by Ahopelto and his group (14) in colorectal cancer, where an elevated FE for TKTL1 was observed on the left side. Ahopelto and his group (14) noted that elevation of TKTL1 in tumor tissue can lead to poor survival in colorectal cancer and thus TKTL1 could serve as a candidate marker to identify patients at risk for recurrence. . Sun with colleagues (15)were reported the high level of TKTL1 gene expression in head and neck squamous cell carcinoma (HNSCC) when comparison with the normal tissues, that is probably due to hypomethylation. The DNA methylation is a type of epigenetic modification that plays an important role in both normal cells and cancer cells. In mammalian cells, the abnormal DNA methylation is a strong cause of many tumors, it has also been revealed that DNA methylation causes mitochondrial dysfunction in cancer cells, plays a regulatory role in processes such as the PPP pathway and glucose formation, and provides raw materials for the rapid proliferation of cancer cells(16)

TABLE 2: The level of Gene Expression Depending on the Age and Location

Clinical Characteristics	No. %	Mean of (FE) TKTL1 gene	P-Value
Age			0.062 N
≥ 50	60)(%30	5.46 ±0.31	
<50	)40(%20	4.89 ±0.25	
Location			0.0388*
Right	46) %(23	5.06±0.24	
Left	27(%54)	4.22 ±0.19	

Relationship of TKTL1 Gene Expression Level with Tumor Type

Table (3) indicates the level of TKTL1 gene expression depending on the tumor type. An increase of FE (4.96) for the TKTL1 gene was observed in Invasive Ductal Carcinomas (IDC) of breast tumors. The FE values were ranged between (0.02-2.66) in 90% (45/50) of patients. The FE expression in Invasive Lobular Carcinoma (ILb) was 1.38, it was ranged between (0.36-2.49) in 10% (50/10) of patients in comparison with 1.48 in control group. The results showed significant differences between IDC and ILb ($p \leq 0.01$).

Our results are consistent with the study by Schmidt and his group(17),they reported that high expression of TKTL1 gene was observed, a significant association in IDC than in normal breast tissue. Rare studies, that deal with identifying the TKTL1gene expression in breast cancer patients, other studies refer to the TKTL1 gene expression in several cancers, high level of TKTL1 was reported in the prostate tumor, as in the study by(18), they suggested that pentose phosphate pathway and the key enzyme TKTL1 were changed in prostate tumors. The TKTL1 gene expression also was elevated in patients with conjunctival carcinoma, the increasing level was associated with poor clinical outcomes, especially in terms of recurrence rates(19). It has been suggested that the anti-transketolas drugs that significantly reduce the growth of cancer cells, acting by inhibiting TKTL1 and preventing the generation of energy for cancer growth, the TKTL1 can be as a potential treatment for cancer.

TABLE 3: The Level of Gene Expression Depending on the Tumor Type

Clinical Characteristics	No. (%)	Mean of FE) TKTL1 gene	P-Value
IDC	) 90(%45	4.96 ±0.31	0.0001**
LC	) 10(%5	1.38 ±0.08	
Control	)100(%25	1.48	

Relationship of TkTL1 Gene Expression Level with Tumor Grade

Table (4) indicates the FE level of TKLL1 gene in breast cancer patients depend on tumor grade. The results showed that the highest rate of FE (4.65) in 78% (39/50) of patients with second and third degree of tumor compared to the FE (1.48) in the control group. A significant association between FE of TKTL1 gene expression and tumor grade ($P \leq 0.01$).The Results of this study inconsistent with the results by(20), as they did not found significant differences for TKTL1 gene expression and grade breast while results of this study keep with study by(21), they found increasing level of FE for TKTL1 gene in Grade II and Grade III ovarian cancer patients. The current study may indicate to the role TKTL1 gene in the development and progression of breast cancer. Detection of TKTL1 expression may be act as a biomarker to detect the breast cancer.

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TABLE 4: The Level of Gene Expression Depending on the Tumor Grade

Clinical Characteristics	No.(%)	Mean of (FE) TKTL1 gene	P-Value
Grade I	) 2(%1	2.49 ±0.12	0.001
Grade II	) 78(%39	4.65 ±0.34	0.0001**
Grade III	) 20(%10	4.57 ±0.25	
Control	) 100(%25	1.48	

P-Value ($P \leq 0.01$)

Relationship of TKTL1 gene expression level with Tumor stage

Table (5) indicates to the level of TKTL1gene expression depending on the tumor stage. The results indicated to increase the mean of FE (6.39) for KTL1gene in 12% (6/50) and (4.90) in 42% (50/21) patients with stage I stage and II respectively .The mean of FE level was 4.52 in 32% (50/16) of patents with stage III breast cancer while the mean of FE level was 1.48 for control group. The results were showed significant differences depend on breast cancer stage ($P \leq 0.01$).

The above results confirm what we mentioned previously regarding the reliability of measuring the level of gene expression of TKTL1 in early detecting of breast tumors due to the presence of a significant difference despite the low percentage. These results are in agreement with the study by Moralli and his group (22) who investigate the FE of the TKTL1 gene in colorectal cancer patients, they found a significant differences between the groups where ($P = 0.00008$), as they refer to high level (32.9) of FE for TKTL1 gene compared to the first stage in breast tumors.

The FE of the TKTL1 gene was elevated in patients with stages I and II of ovarian cancer with statistical differences(21).

The present results dis agree with the other studies (13)(20)

It was reported that the TKTL1 gene affects the overall Tranketolas activity and cell proliferation in human hepatoma cells, indicating that TKTL1 plays an important role in glucose metabolism in tumors and may become a new target for tumor gene therapy, the most effective way to prevent tumor proliferation should be to prevent the generation of energy and ribose for tumor growth (23). A therapeutic intervention that targets the tumor metabolism of malignant endometrial cells has the potential to be effective without harming benign cells, so TKTL1 could serve as a target for future cancer therapy(24).

TABLE 5: The Level of Gene Expression Depending on the Tumor Stage

Clinical Characteristics	No.(%)	Mean of FE TKTL1 gene	P-Value
Stage I	) 12(%6	±0.37 6.39	0.0001**
Stage II	) 42(%21	4.90 ±0.22	
Stage III	) 23(%16	4.52 ±0.19	
Stage IV	) 14(%7	2.37 ±0.15	
Control	) 100(%25	1.148	

CONCLUSIONS

High level of TKTL1gene expression gene was found in Iraqi females with breast cancer with significant relation depending on site, type, second grade, and stage of breast cancer on the right side. The study indicated that the expression of TKTL1gene maybe act as a biomarker for early detection of breast cancer.

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