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Role of alpha and gamma Klotho genes in the development of differentiated thyroid carcinoma on top of goiter

Research Article

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Abstract: Background: Differentiated thyroid carcinoma (DTC) is the most common malignant tumor of the endocrine system. Our study is aimed to investigate the role of the α- and γ-Klotho genes in the development and progression of DTC.
Methods: The expression of the α- and γ-Klotho genes was assessed by quantitative real-time polymerase chain reaction (RT-qPCR) in thyroid tissues of 40 DTC patients and 40 age- and sex-matched subjects diagnosed as goiter and included as a control group. The demographic, laboratory and clinicopathological data of the subjects were reviewed to detect their association with the Klotho genes.
Results: The α-Klotho gene expression was statistically significantly lower in DTC tissues compared to goiter tissues ($p < 0.001$). However, there was no statistically significant association between the γ-Klotho gene expression and DTC ($p = 0.643$). Receiver operating characteristic (ROC) curve analysis showed the diagnostic value of the α-Klotho mRNA levels, by providing an AUC of 0.954 value (95% CI = 0.910–0.999; $p < 0.001$). Lower α-Klotho gene expression associated significantly with higher stages of DTC ($p = 0.026$). Logistic regression analysis declared that low α-Klotho mRNA expression was demonstrated to be a significant predictor for the likelihood of DTC on top of goiter ($p = 0.001$).
Conclusions: Our study showed the role of the α-Klotho gene as a potential novel biomarker for discrimination between DTC and goiter tissues. Low α-Klotho mRNA expression was found to be a significant predictor for the likelihood of DTC on top of goiter, as well as higher stages of this tumor.

Keywords: *Differentiated thyroid carcinoma • α-Klotho • γ-Klotho • gene expression • biomarker*

1. Introduction

Thyroid carcinoma (TC) is the most common endocrine tumor, accounting for 3.1% of worldwide cancer incidence[1]. In 2022, about 43,800 new cases in the United States were diagnosed with TC[2]. In Egypt, TC is the fifth most frequent cancer in females accounting for 3.6% of all malignancies in women[3]. It is classified as differentiated papillary (PTC), follicular (FTC), and undifferentiated or anaplastic (ATC)[4]. The incidence of DTC has been growing noticeably over the past 20 years worldwide, and is anticipated to be the fourth most common malignancy by 2030[5]. TC is initiated by genetic alterations and epigenetic changes in oncogenes or tumor suppressor genes[6]. These genetic alterations particularly engage a small set of genes whose protein products are typically members of the mitogen-activated protein kinase (MAPK) and PI3K/PTEN/AKT/mTOR signaling pathways[7].

The alpha Klotho (α-Klotho, KL) was identified in 1997 as an antiaging gene[8, 9]. Two other related genes, beta Klotho (β-Klotho) and gamma Klotho (γ-Klotho), have been identified as Klotho family members[10]. The α-Klotho gene spans 50 kb and is located on chromosome 13q12[8]. It encodes a type 1 single-pass transmembrane glycoprotein containing two large extracellular domains, KL1 and KL2, with a short single transmembrane and a short intracellular domain in its C-terminus. The γ-Klotho

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gene is located on chromosome 15q22.31 with 14 exons. It encodes a smaller single-pass transmembrane glycoprotein, with a small intracellular domain and a family 1 glycosidase-like extracellular KL1 domain[8, 10, 11].

The Klotho proteins are cofactors of fibroblast growth factors (FGF) 19, FGF21, and FGF23, which control specific metabolic activities of different tissues[11, 12]. The α-Klotho gene has been associated with several human malignancies including hepatocellular, renal cell, colorectal, pancreatic, breast, and lung cancers[13, 14].The α-Klotho functions as a tumor suppressor gene mainly by regulating oxidative stress, insulin/IGF-1, FGF23, p53/p21 and Wnt signaling pathways[13, 15]. On the other hand, there are limited researches about the correlation between the γ-Klotho gene and cancer[11, 16].

The association between the α-Klotho gene and TC remains unclear. Up till now, there has been no study that investigated the role of the γ-Klotho gene in TC. The aim of this study was to assess the α-Klotho and γ-Klotho gene expressions in patients with DTC, which may help better understanding of the pathogenesis of this tumor.

2. Patients and methods

2.1. Patients, sample collection, and preservation

The present case control study was carried out in Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt. This study included 40 DTC patients (31 PTC and 9 FTC patients) in addition to 40 age- and sex-matched subjects diagnosed as goiter and enrolled as a control group. All subjects were selected from Mansoura Oncology Center, Mansoura University, Mansoura, Egypt between December 2019 and March 2021. Informed consents were obtained from all participants in the study. The study protocol was approved by Institutional Research Board (IRB) of Mansoura Faculty of Medicine (IRB code MDP.19.09.25).

The included patients were diagnosed with primary DTC by preoperative palpation, fine-needle aspiration cytology (FNAC), and US. The diagnosis was confirmed by intraoperative rapid pathological and postoperative pathological examination. For all patients, full medical history was taken and careful clinical examination, including size and consistency of thyroid gland, assessment of lymph node, and body mass index (BMI) was done.

Patients with other primary malignancies or who previously underwent hemi-thyroidectomy, anti-TC chemotherapy, radiotherapy, or radioactive iodine treatment before surgery were excluded from this study. All laboratory investigations, including complete blood count (CBC), serum total triiodothyronine (T3), total thyroxine (T4), and thyroid stimulating hormone (TSH) were done in Mansoura Oncology Center in a time frame of less than 3 months preoperatively. The thyroid gland was evaluated by US characteristics, including margins, calcifications, and echogenicity.

Thyroid tissue specimens were obtained at the time of surgery from patients and controls and dissected immediately after surgery. The tumor tissues were collected from the center of the suspected viable cancer tissue[17]. The other part of the tissue samples was transported to the Pathology Department for confirmation of histopathological diagnosis and collection of other clinicopathological data. According to the American Joint Committee on Cancer (AJCC), 2010 recommendations, the TNM staging was accomplished (32 cases were stage I, three cases were stage II, one case was stage III, and four cases were stage IVA cancers).

To preserve RNA integrity, the fresh tissue samples were completely submerged as quickly as possible in an appropriate volume of RNAlater RNA Stabilization Reagent, approximately 10 µl of the reagent per 1 mg of tissue (Qiagen, Germany, Cat. No 76104). The samples were transported on ice and stored at 4°C for at least 24 hours, then stored at −80°C for subsequent total RNA extraction.

2.2. RNA extraction and gene expression

Referring to the modified Chomczyński and Sacchi's method[18], total RNA was extracted from thyroid tissue samples, using QIAzol Lysis Reagent kit (Qiagen, Germany, Cat No 79306) according to the kit's protocol. The integrity of RNA was evaluated by loading RNA samples on agarose gel electrophoresis. The RNA concentration and purity of the samples were assessed using the NanoDrop 2000c Spectrophotometer (Thermo Scientific, USA). The purity of RNA was assessed in each sample via two optical density (OD) ratios (A260/A280 and A260/A230). The RNA samples with 1.8 to 2.0 A260/A280 ratios were used. The isolated RNA was then stored at −80°C for subsequent reverse transcription.

Complementary DNA (cDNA) was synthesized using COSMO cDNA synthesis kit (Willowfort, COSMO cDNA synthesis kit, Birmingham Research and Development Park, Birmingham, WF-10205002), in accordance with manufacturer's instructions. The cDNA was synthesized

using the thermal cycler (Applied Biosystem, Waltham, Massachusetts, USA) with the following program of 5 min at 25°C, 15 min at 45°C, and 5 min at 85°C. The synthesized cDNA samples were stored at −20°C.

The RT-qPCR assays were carried out on the 7500 Real Time PCR System, Applied Biosystem, USA, with HERAPLUS SYBR® Green qPCR Master Mix (2X), Birmingham Research and Development Park, Birmingham, WF10308001, and gene-specific real-time qPCR primers. It was performed according to the method described by Freeman et al.[19]. Each 20 µL reaction mix contained 10 µL of HERAPLUS SYBR® Green qPCR Master Mix (2X), 2 µL of the synthesized cDNA, 1 µL of forward primer, 1 µL of reverse primer, and the remaining 6 µL was RNase free water.

The primers for the α-Klotho and γ-Klotho genes were chosen from NCBI databases [http://www.ncbi.nlm.nih.gov/tools/primer-blast]. The primer sequences were checked for the melting temperature, product length, GC ratio, 3' complementarity, and self-complementarity using Primer3 v.4.1.0 software [http://primer3.ut.ee/]. The forward primer sequence of the α-Klotho gene was 5′-CTGGATCACCATCGACAACCC-3′ and the reverse primer was 5′-GACACCTGACCTCCCTGAGT-3′ for a product size of 186 bp. The forward primer sequence of the γ-Klotho gene was 5′-GGCTCTGACTACCAGCGACT-3′ and the reverse primer was 5′- CACCAGCAGTAGCATCCACA-3′ for a product size of 125 bp. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene, and its primers were selected based on published sequences[20]. The forward primer sequence of the GAPDH gene was 5′-GTCAAGGCTGAGAACGGGAA-3′ and the reverse primer was 5′-AAATGAGCCCCAGCCTTCTC-3′ for a product size of 158 bp.

The real-time PCR reactions were performed with the following thermal cycling program of 2 min at 95°C, followed by 40 cycles of denaturation at 95°C for 10 s and annealing/extension at 60°C for 30 s. The analysis of melting curve of all the reactions was performed for evaluation of the specificity of the products.

2.3. Interpretation of results

Relative quantification (RQ) of mRNA expression was estimated using the comparative threshold method (ΔΔCt)[21]. The data were presented as RQ of the target mRNA, normalized as regards the mRNA of the reference gene GAPDH and in respect to the control samples. The fold change was calculated using the equation $RQ = 2^{-\Delta\Delta Ct}$.

2.4. Statistical analysis

The collected data were introduced to a PC using Statistical Package for Social Science (SPSS) Version 25. Qualitative data were expressed as count and percent. Quantitative data were initially tested by Kolmogorov–Smirnov and Shapiro–Wilk's, then expressed as mean ± standard deviation (SD) for parametric numerical data or median and interquartile range (IQR) for nonparametric numerical data. Qualitative data were compared via Chi-square test (or Fisher's exact test), whereas quantitative data were compared via independent samples t-test or nonparametric Mann–Whitney U test. Receiver operating characteristic (ROC) curve analysis was used to determine the discrimination accuracy of the diagnostic test to distinguish between DTC and goiter[22]. Comparisons of area under ROC curve (AUC) were performed. Logistic and ordinal regression analyses were used for prediction of risk factors, using generalized linear models. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated. The results were considered statistically significant if *p*-value ≤ 0.05 for any used test.

3. Results

3.1. Characteristics of DTC patients and controls

Routine demographic and laboratory data of the patients and controls are presented in Table 1. DTC patients had a statistically significantly lower BMI than the controls ($p = 0.008$). The serum TSH level was statistically significantly higher in patients with DTC than in controls ($p = 0.016$).

3.2. Comparison of α-Klotho and γ-Klotho gene expressions among DTC patients and controls

The α-Klotho gene expression was statistically significantly lower in DTC patients (median: 0.34; IQR: 0.22–0.54) compared to controls (median: 0.96; IQR: 0.83–1.19; $p < 0.001$). However, there was no statistically significant difference between the case (median: 0.95; IQR: 0.63–1.35) and the control groups (median: 1; IQR: 0.80–1.30; $p = 0.643$) regarding the γ-Klotho gene expression.

Table 1: Demographic and laboratory parameters of the studied groups.

Parameter	Group		*p* value
	Case (DTC) (*n* = 40)	Control (goiter) (*n* = 40)	
Age (years)	41.08 ± 11.3	41.08 ± 11.3	*1.000
Sex:			
Male	9 (22.5%)	9 (22.5%)	**1.000
Female	31 (77.5%)	31 (77.5%)	
BMI (kg/m²)	30.30 ± 6.2	33.80 ± 5.4	***0.008**
Serum total T3 (µg/dL)	1.27 ± 0.4	1.23 ± 0.3	*0.456
Serum total T4 (µg/dL)	7.24 (6.50-8.25)	7.85 (6.30-8.85)	***0.528
Serum TSH (mIU/L)	1.10 (0.66-1.58)	0.82 (1.03-2.45)	****0.016**

P-value by *Independent samples t-test (data are presented as mean ± SD); p-value by **Chi-square test (data are presented as count and percent); *p*-value by ***Mann–Whitney U test (data are presented as median and IQR).

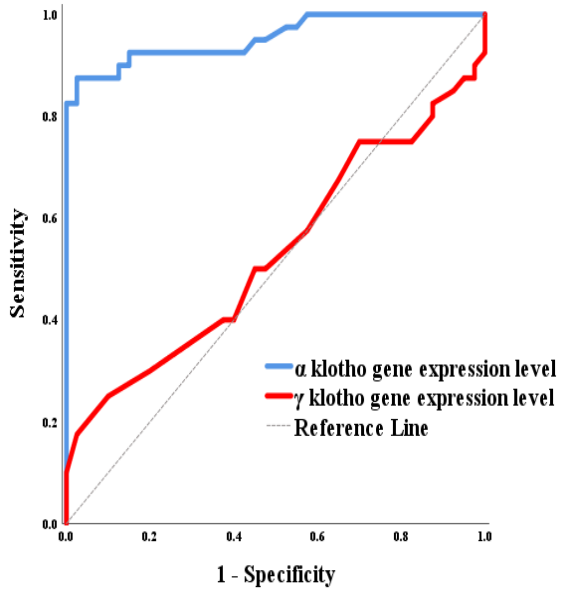

Figure 1: ROC curve analysis of the α-Klotho and γ-Klotho genes for the diagnosis of DTC versus goiter.

3.3. ROC curve analysis of the α-Klotho and γ-Klotho gene expressions in the diagnosis of DTC versus goiter

ROC curve analysis showed that the α-Klotho mRNA levels can discriminate between malignant and benign thyroid tissues by providing an AUC of 0.954 value (95% CI = 0.910–0.999; $p < 0.001$). However, ROC curve analysis of the γ-Klotho mRNA levels could not discriminate between malignant and benign thyroid tissues by providing AUC of only 0.530 (95% CI = 0.401–0.659; $p = 0.644$) (Figure 1).

With a specificity of 97.5% and a sensitivity of 87.5%, the α-Klotho cutoff value of ≤ 0.68 showed a significant diagnostic accuracy of DTC. However, the γ-Klotho cutoff value of ≤ 0.93 did not show significant diagnostic accuracy of the tumor with a specificity of only 55.0% and a sensitivity of only 50.0%.

3.4. Association between DTC patients' demographics and clinicopathological parameters and the two categories of the α-Klotho gene expression

DTC patients were classified into high and low α-Klotho gene expression groups, according to the cutoff value of the α-Klotho mRNA level (≤0.68). Low expression of the α-Klotho gene was identified in 35 of the 40 samples of the tumor tissues. The low α-Klotho expression group had larger tumor size ($p < 0.001$) and multifocality tendency ($p = 0.031$). However, there was no significant correlation between the α-Klotho expression and the other studied variables ($p > 0.05$; Table 2).

3.5. Correlations of the α-Klotho gene expression with γ-Klotho gene expression and with different parameters

There was a statistically significant inverse correlation between the α-Klotho gene expression and the age of the studied groups ($p = 0.037$), the stage of the DTC ($p = 0.026$), as well as the tumor size ($p < 0.001$). However, there were no statistically significant correlations between the α-Klotho gene expression and the other studied parameters ($p > 0.05$; Table 3).

3.6. Correlations of the γ-Klotho gene expression with different parameters

There were no statistically significant correlations between the γ-Klotho gene expression and the studied parameters ($p > 0.05$; Table 4).

3.7. Prediction of the likelihood of DTC

Logistic regression analysis revealed that low α-Klotho mRNA expression was demonstrated to be significant predictor for the likelihood of DTC on top of goiter ($p = 0.001$; Table 5). Univariate ordinal regression analysis was performed. It revealed that the female gender ($p = 0.025$), high-serum total T4 ($p = 0.010$),

Table 2: Association between the demographics and the clinicopathological parameters and the two groups of the α-Klotho gene expression.

Parameter		α-Klotho gene expression (Fold change)		p
		High (>0.68) (n=5)	Low (≤0.68) (n=35)	
Age	<45years	4 (80%)	21 (60%)	**0.388
	≥45years	1 (20%)	14 (40%)	
Sex	male	1 (20%)	8 (22.9%)	**0.886
	female	4 (80%)	27 (77.1%)	
BMI (kg/m2)		30.3±5.4	30.3±6.4	*0.988
Tumor size (cm)		0.94±0.4	2.91±0.9	*<0.001
LNM	absent	3 (60.0%)	17 (49.0%)	**0.633
	present	2 (40.0%)	18 (51.0%)	
Lateral LNM	absent	3 (60.0%)	22 (62.9%)	**0.902
	present	2 (40.0%)	13 (37.1%)	
Lymphovascular permeation	absent	4 (80.0%)	20 (57.1%)	**0.631
	present	1 (20.0%)	15 (42.9%)	
Lymphocytic infiltration	absent	4 (80.0%)	30 (85.7%)	**0.738
	present	1 (20.0%)	5 (14.3%)	
Extrathyroidal invasion	absent	5 (100.0%)	27 (77.1%)	**0.232
	present	0 (0.0%)	8 (22.9%)	
Capsular invasion	absent	5 (100.0%)	23 (65.7%)	**0.298
	present	0 (0.0%)	12 (34.3%)	
Multifocality	absent	5 (100.0%)	17 (48.6%)	**0.031
	present	0 (0.0%)	18 (51.4%)	
Pathological type	papillary	4 (80.0%)	27 (77.1%)	**0.525
	follicular	1 (20.0%)	8 (22.9%)	
Calcification	absent	3 (60.0%)	16 (45.7%)	**0.550
	present	2 (40.0%)	19 (54.3%)	
Margin	ill-defined	0 (0.0%)	15 (42.9%)	**0.137
	well-defined	5 (100.0%)	20 (57.1%)	
Stage	I	5 (100%)	27 (77.1%)	**0.699
	II	0 (0%)	3 (8.6%)	
	III	0 (0%)	1 (2.9%)	
	IVA	0 (0%)	4 (11.4%)	

p-value by *Independent samples t-test (data are presented as mean ± SD); *p*-value by **Chi-square test (data are presented as count and percent).

Table 3: Correlations of the α-Klotho gene expression with γ-Klotho gene expression and with different parameters.

α-Klotho gene expression		
Parameter	Correlation coefficient	*p*-value
γ-Klotho gene expression	-0.065	0.565
Age	-0. 233	**0.037**
BMI	-0.210	0.194
Serum total T3	0.084	0.606
Serum total T4	0.101	0.534
Serum TSH	-0.258	0.108
Stage	-0.346	**0. 026**
Tumor size	-0.898	**<0.001**

p-value by Spearman's correlation coefficient test.

Table 4: Correlations of the γ-Klotho gene expression with different parameters.

γ-Klotho gene expression		
Parameter	Correlation coefficient	*p*-value
Age	-0.028	0.866
BMI	-0.254	0.114
Serum total T3	0.186	0.250
Serum total T4	0.244	0.130
Serum TSH	-0.156	0.338
Stage	-0.104	0.525
Tumor size	-0.089	0.586

p-value by Spearman's correlation coefficient test.

Table 5: Logistic regression analysis to predict the likelihood of DTC on top of goiter.

Parameter	Crude OR	95% CI	*p*-value
BMI	0.998	0.99-1.006	0.626
Serum total T3	1.023	0.83-1.26	0.831
Serum total T4	1.004	0.97-1.039	0.836
Serum TSH	1.063	0.95-1.19	0.285
α-Klotho gene expression	0.506	0.343-0.746	**0.001**
γ-Klotho gene expression	1.059	0.893-0.1.256	0.508

p-value by simple logistic regression analysis (crude OR: crude odds ratio; 95% CI: 95% confidence interval).

Table 6: Predictors of higher stage of DTC.

Predictor	Adjusted OR (95% CI)	*p*-value
Gender (female)	2.2395(0.747-7.675)	0.142
Serum total T4	1.220(0.956-1.56)	0.110
Serum TSH	0.812(0.587-1.125)	0.211
α-Klotho gene expression	0.671(0.246-0.835)	**0.037**

p-value by multivariate ordinal regression analysis (adjusted OR: adjusted odds ratio; 95% CI: 95% confidence interval).

high-serum TSH ($p = 0.024$), and low α-Klotho mRNA expression ($p < 0.001$) could be significant predictors for the probability of higher stage of DTC.

Multivariate ordinal logistic regression was performed to ascertain the effects of the female gender, high-serum total T4, high-serum TSH and low α-Klotho mRNA expression on the likelihood of higher stage of DTC. Patients with higher α-Klotho mRNA expression had a lower odds (0.671) to exhibit higher stage of DTC ($p = 0.037$; Table 6). Higher α-Klotho mRNA level appeared as a protective factor against higher DTC stage.

4. Discussion

Since Wolf et al.[23] had discovered that the α-Klotho had an inhibitory effect on breast cancer, the role of this gene in the pathogenesis, development, and prognosis of cancer has received more and more attention. It has been shown that the α-Klotho functions as a tumor suppressor gene in most malignancies, such as lung, gastric, pancreatic cancers, and melanoma[24-26]. However, some controversial results have also been reported, such as the effects of the gene on the angiogenesis and antiapoptosis that may play a part in the progression of the ovarian cancer[27].

In the present study, we evaluated the expression levels of the α-Klotho and γ-Klotho genes in DTC against goiter in the Egyptian population. We reported that the α-Klotho gene expression was statistically significantly decreased in DTC patients compared to controls ($p < 0.001$). In line with our findings, Pawlikowski et al.[28] had showed that lowered expression of the α-Klotho gene had been involved in the process of the thyroid neoplasia. Dai et al.[29] had demonstrated that overexpression of the α-Klotho gene in the follicular thyroid cancer cell lines resulted in a decreased capacity for cell proliferation and an increased capacity for cell apoptosis ($p < 0.05$). Wu et al.[30] had reported a significant decrease of the α-Klotho gene expression in the papillary thyroid cancer cells ($p < 0.05$).

Goiter can progress to TC in approximately 4–14% of cases[31]. Thus, advances in molecular biology are essential to provide new insights into diagnosis of goiter and prediction of malignant transformation[32]. The ROC curve analysis in our study indicated that the mRNA levels of α-Klotho gene with cutoff value of ≤0.68 had the potential to discriminate between benign and malignant thyroid tissue specimens with a specificity of 97.5% and a sensitivity of 87.5%. Our research showed that the α-Klotho can be used as a novel molecular marker to discriminate between DTC and goiter tissues.

Our study revealed that DTC patients with lower α-Klotho mRNA levels had larger tumor size ($p < 0.001$) and exhibited multifocality ($p = 0.031$). The correlation analysis in our study showed the statistically significant inverse relationship between the α-Klotho gene expression and the age of the studied groups ($p = 0.037$). The expression levels of α-Klotho mRNA and its protein decrease by aging, as the gene controls numerous signaling pathways concerned with aging processes, for example, Wnt pathway, insulin signaling, and phosphate homeostasis[33, 34].

Also, we reported the inverse correlation between the α-Klotho gene expression and the stage of DTC ($p = 0.026$), as well as the tumor size ($p < 0.001$). Li and his colleagues[35] had injected the mice with transduced osteosarcoma cells with overexpressed α-Klotho to evaluate the effect of α-Klotho on tumor growth. After 4 weeks, they had found that α-Klotho overexpression significantly diminished the tumor weight and volume in the mice model of osteosarcoma, compared to controls. In disagreement with our results, Huang et al.[26] had observed that the α-Klotho gene expression did not significantly correlate with the tumor diameter among the hepatocellular carcinoma patients ($p = 0.208$).

Zhou *et al.*[36] had showed that lower expression level of the α-Klotho gene was associated significantly

with stage III or IV of diffuse large B-cell lymphoma, compared with stage I or II (p = 0.002). Also, Huang et al.[26] had showed that the α-Klotho expression level was significantly negatively associated with the clinical stage of the hepatocellular carcinoma, stage I and II versus stage III and IV (p = 0.017).

There are few reports about the relationship between the γ-Klotho gene and cancers. For example, its effect on bladder cancer invasion and progression, colon cancer cell proliferation and its association with poor triple-negative breast cancer prognosis[11, 16].

In our study, we did not detect significant difference between the DTC patients and controls regarding the γ-Klotho gene expression (p = 0.643). Contrary to our results, Trošt et al.[16] had reported that the γ-Klotho gene was significantly upregulated in breast cancer relative to normal breast tissue (p = 0.0034). The high expression level of the γ-Klotho correlated with poor progression of the disease and the in vitro analysis revealed that the gene was an essential factor for cell survival. The research of Hori et al.[11] in human bladder carcinoma had revealed that the exogenous γ-Klotho treatment enhanced the ability of the tumor to invade, migrate, and colonize in the in vitro study. Also, Onishi et al.[37] had indicated that the γ-Klotho gene has an important role in prostate carcinogenesis.

In our study, there were no statistically significant correlations between the γ-Klotho gene expression and different parameters, as age, BMI, serum T3, T4, TSH, tumor stage, and size (p > 0.05). In contrast, the γ-Klotho siRNA treatment had a significant suppression of the tumor growth of bladder cancer in vivo. Also, in vitro study that had used exogenous γ-Klotho treatment had identified higher tumor progression ability, including cell invasion, migration, and colony formation[11]. These findings had suggested that the γ-Klotho may have a role in tumor growth, by enhancing the cell cycle and suppressing the apoptosis.

Trošt and his colleagues[16] had reported higher stage and worse progression in patients with triple-negative breast cancer, who expressed higher γ-Klotho expression. Also, Su et al.[38] had identified the aggressive behavior of the γ-Klotho, as its high expression predicted poor prognosis for glioma patients.

These different findings in our study and the others may be due to our small sample size, different tumor type, ethnicity, or presence of other factors.

To the best of our knowledge, the current study is the first one as regards the relationship between the γ-Klotho gene and DTC. The direct association between the γ-Klotho as a cofactor in FGF signaling pathway could not be illustrated in this research. Absence of significant association between the γ-Klotho gene expression and DTC in our study may be due to small sample size of our studied groups and population differences.

The logistic analysis of the current study revealed that low expression of α-Klotho mRNA, with a cutoff value ≤0.68 and quantified via qRT-PCR, was a significant predictor for the likelihood of DTC on top of goiter (p = 0.001). The ordinal regression analyses revealed that the low α-Klotho mRNA expression was a significant predictor for the likelihood of higher stage of DTC.

In conclusion, our results declared the tumor suppressor role of the α-Klotho gene in DTC and its importance as a promising novel biomarker for DTC. This also may provide opportunities for development of new Klotho-based therapies. To the best of our knowledge, there has been no study investigating the role of the γ-Klotho gene in DTC before. In our study, there was no significant difference between DTC and goiter as regards the γ-Klotho gene expression.

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Conflict of interest

The authors report no conflict of interest regarding this publication.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, methodology, and manuscript writing were performed by Wesam S. El-Saeed and Marwa H. Elnagdy. Samples collection and pathological reports analysis were performed by Mahmoud A. Abd elghaffar. Methodology supervision, manuscript editing, and revision were performed by Ayman El Baz and Mohammed A. Zahran. All authors read and approved the final manuscript.

Ethics approval

The study protocol was approved by Institutional Research Board (IRB) of Mansoura Faculty of Medicine (IRB code MDP.19.09.25).

Informed consent

Informed consent was obtained from all individual participants included in the study.

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