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Downregulation of *TPO* gene and it's related with Iodine deficiency in women with hypothyroidism

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Abstract:

The purpose of this study was to detect the levels gene expression of *TPO* and its role iodine deficiency in patients with hypothyroidism. Cases collected during the period from April 2018 to December 2018. All cases were obtained from Al-Zahra Teaching Hospital in Wasit Province/Iraq. Sixty women with hypothyroidism aged from(20-70) years were involved in this study. The results showed the highest percentage of incidence in age groups (41-50) and (51-60) years were recorded 38.33% and 31.33% respectively. Hormonal assay showed a significantly increase (11.34±0.27) in TSH and significantly decrease (0.87±0.03), (93.41±5.81) in T3 and T4 levels respectively. Gene expression of *TPO* appeared to downregulation in hypothyroidism patients were the *TPO* gene folding recorded (0.42±0.06) when compared to folding of *TPO* gene in healthy control group(1±0.00). In conclusion, this study confirm the downregulation of *TPO* gene was related with Iodine deficiency in patient with hypothyroidism.

Keywords: Hypothyroidism , Thyroid peroxidase , *TPO* gene

1- INTRODUCTION:

Hypothyroidism is the most common pathological dyshormonogenesis caused by a primary process that produces insufficient amounts of thyroid hormone[1]. Thyroid disease interferes with the physiology of the reproduction; both hyperthyroidism and hypothyroidism have significant effects on estradiol and androgen metabolism, menstrual function and fecundity [2]. There are relationship between genetic alternation of *TPO* gene and induction of polycystic ovary syndrome [3]. The synergistic of hypothyroidism and hyperprolactemia in women leading to defect in menstrual cycle and ovulatory status in women with reproductive age [4].The human *TPO* gene (NG_011581.1), which localizes on chromosome 2p25, is divided into17exons and covers approximately150 kb of DNA [5]. More than 61 mutations in the *TPO* gene have been recorded of which are missense mutations [6]. The first mutation of the *TPO* gene was identified in (1992), it was a GGCC duplication in exon 8 at position 1227, which is a common *TPO* mutation subsequently found in patients originating from many countries [7]. A number of single nucleotide polymorphisms (SNP) located in exons 2,7,8,10,11,12 and 15, have been reported for the *TPO* gene. Interestingly, beside exonic variants, a 50 bp repeat in intron 10 has also been described as highly polymorphic, with a number of repeats varying from 9 to 31, but not influencing the alternative splicing of exon 10 [8]. Therefore, the aim of this study was to investigate the levels of *TPO* gene in women with hypothyroidism.

2. MATERIAL AND METHODS:

2.1. Subjects

Two study groups have been investigated in this study:

2.1.1. Patients group:

Sixty women with hypothyroidism. Cases collected during the period from April 2018 to December 2018. All cases were obtained from Al-Zahra Teaching Hospital in Wasit Province/Iraq. Patients aged from (20-70) yrs were included in this study.

2.1.2. Healthy control group:

Healthy group consists of twenty healthy women of different ages.All individuals were chosen dependent on the next criteria: age 20 to 45 years, No history of endocrine disease and no use of medication

2.1.3. Blood sampling:

Venous blood sample (5 ml) was collected from each and healthy control. Each blood sample was divided into two tubes: Plain tubes for hormonal analysis and Tizol tubes for gene expression.

2.2. Hormonal assay:

The serum obtained by putting the blood samples in a plain tube and allowed to clot at 37C˚ for 30 minutes before centrifugation. The tubes centrifuged at 5000 rpm for 5 minutes, serum was kept in freezer until used. Hormonal assay was performed by using Addendum-Minii VIDAS apparatus (VIDAS) 12 mode l0/BioMerieux Company/ France ,through an enzyme linked fluorescent assay(ELFA) technique. The instrument in relation to the calibration curve stored in memory, then automatically calculates results. The procedure was applied to T3, T4and TSH, hormones are determined by using VIDAS T3, VIDAS T4 and VIDAS TSH kits respectively. The values of hormonal assays for the subjects were compared with the next normal range: T3= 0.95-2.5n.mol/L, T4= 60-120n.mol/L and TSH = 0.25-5µ.IU/ml.

2.3. Gene Expression:

Total RNA of all samples was extracted using the TRIzol®LS Reagent according to the manufacturer's instructions. Total RNA was reversely transcribed to complementary DNA (cDNA) using WizScript™ RT FDmix Kit. The procedure was carried out in a reaction volume of 20µl. The reveres transcription step was carried out one cycle using the next program: 25°C for 10 min,42 °C for 10 min,85°C for 5 min and 4°C to the end of the run. The expression levels of *TPO* gene were estimated by quantitative real time-PCR (qRT-PCR). To confirm the expression of target gene EVA Green was used. The mRNA levels of reference gene Glyceraldehyde 3-

phosphaee dehydrogenase (*GAPDH*) were amplified and used to normalize the mRNA levels of the *TPO* gene. qRT-PCR reaction was performed using specific primers were designed for the exon9 *TPO* gene. Lyophilized primers were dissolved in a free DNase/R Nase water to give a final concentration of (100 pmol/µl) as stock solution, to prepare 10µM concentration as work primer resuspended 10 pmol/µl in 90 µl of demonized water to reach a final concentration 10µM as work solution, the program of the reaction was : Initial denaturation: 95°C for 5 min (on cycle), Denaturation: 95°C for 40 sec, annealing: 59°C for 40 sec, Extension :72 °C for 1 min , the run carried out with 35 cycles then holding with 4 °C for 1 cycle). The sequences of GAPDH primers was F:5′-AACTTTGGCATTGTGGAAGG-3′, R:5′-ACACATTGGGGGTAGAACA-3[9] and *TPO* gene was (F:5-TGCTTTTCCTATCTGCACAGATCACCC-3, R: 5-ACCAGCTGCAGGGACCGCACTCA-3[3]**.**

2.4. Statistical analysis:

ΔCT and ΔΔCT were calculated according to Livak and Schmittgen equation [10]. The means were conducted according to Statistical Analysis System-SAS [11] to measure the effect of different factors in studying the parameters. Least significant difference (LSD) test was used to compare between means. The means and standard deviations were recorded for each sample variables included Ct values and gene expression levels. This included values of housekeeping gene and test gene. P value for all tests was considered high significant if <0.01

3. RESULTS:

3.1. Distribution of hypothyroidism according to age:

This study involved 60 hypothyroidism women with age ranged between 20-70 years. The highest percentage of incidence as follow: The age (41-50) years with percentage (38.33%) and the age group (51-60) years that recorded (31.33%) followed by (18.33%) in age group (31-40) years. Table (1) shows the age and and percentage of incidence with hypothyroidism.

3.2. Hormonal Profile:

The variations of mean values of TSH,T3 and T4 levels were represented in table (2). Patients with hypothyroidism showed to have a significantly increased levels of TSH when compared with healthy control group, the concentration of T3 hormone was significantly increased if thy compared with control group and there were significantly decreased in T4 hormone levels than the normal range values.

Table (1): Age and percentage of hypothyroidism.

Age group	Number of patients	Percentage (%)
20-30	5	8.33
31-40	11	18.33
41-50	23	38.33
51-60	19	31.67
61-70	2	3.33
Total	60	100

Table (2): Levels of TSH , T4 and T3 in patients with hypothyroidism and healthy control group .

Hormones	Mean ± SE		LSD
	Control	Hypothyroidism	
TSH µIU/ml	4.11 ± 0.15	11.34 ± 0.27	2.733 **
T3 n. mol/L	1.55 ± 0.07	0.78 ± 0.03	0.569 **
T4 n. mol/L	76.07 ± 4.63	93.41 ± 5.81	9.251 **
** (P<0.01).			

TSH : Thyroid-Stimulating Hormone , T3: Triiodothyronine , T4: Thyroxin ,SE:
Standard Error , LSD: Least Significant Differences .* (P<0.05) .

3.3. Quantitative Real-Time PCR results:

3.3.1. Housekeeping gene Expression:

There were no significant differences of Ct value of housekeeping gene (*GAPDH*) in subjects and healthy control group (1±0.00)**.** The housekeeping gene expression used in the present study as shown in table (3).

3.3.2. *TPO* gene expression:

Expression of the *TPO* gene was highly significant decreased (p<0.01) in Hypothyroidism patients when compared to the healthy control group as shown in table (4).

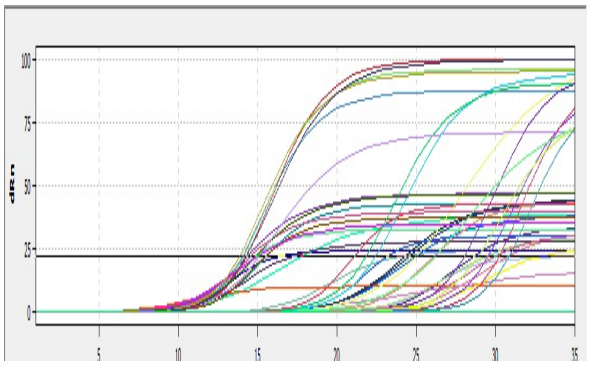
Table (3): Comparison of *GAPDH* gene fold expression between study groups.

Group	Mean Ct of GAPDH	2^{-Ct}	experimental group/ Control group	Mean fold of *GAPDH* Expression
Hypothyroidism	20.753	5.659E-07	5.659E-07 /5.309E-07	1.07
Control	20.845	5.309E-07	5.309E-07/5.309E-07	1.00
LSD value	---	---	---	0.169 NS
NS: Non-Significant.				

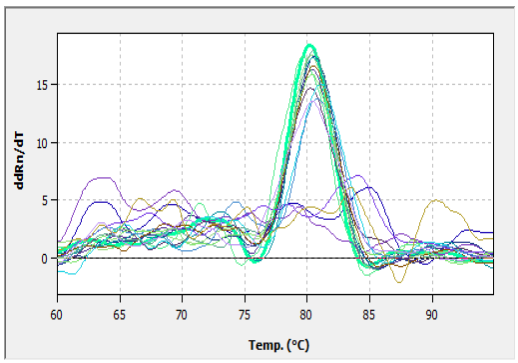
Table (4): Fold of *TPO* gene expression. Depending on $2^{-\Delta Ct}$ Method.

Groups	Means Ct (*TPO*)	Means Ct (*GAPDH*)	ΔCt= Means Ct (*TPO*)- Means Ct (*GAPDH*)	$2^{-\Delta Ct}$	Experimental group/ Control group	Folding
Hypothyroidism	24.87	20.23	+4.64	0.400	0.400/0.095	0.42 ± 0.06
Control	22.53	19.13	+3.4	0.095	0.095/0.095	1 ± 0.00
LSD value	---	---	---	---	---	0.285 **
** (P<0.01).						

Figures (1,2) show the amplification plots and melting curve respectively for *TPO* gene



Figure(1):*TPO* and *GAPDH* genes amplification plots by qPCR .Ct values of *TPO* ranged from 25.34 to 30.13 and GAPDH Ct value ranged from 8.23 to 18.22 .The photograph was taken directly from Qtower2.0/2.2,



Figure(2):*TPO* and *GAPDH* genes melting curves by qPCR Samples included all study groups. Melting temperature ranged from 78°C to 84°C, No primer dimer could be seen. The photograph was taken directly from from Qtower2.0/2.2

4. DISCUSSIONS:

Most of thyroid disorders associated with age and gender is hypothyroidism, which is more common in older women, and (10) times more common in women than in men [12,13]. Community studies of hypothyroidism prevalence determining thyroid disease is much more common in females than in males and the hypothyroidism frequency increases with age 2% to more than 20% of older age [14,15]. The variations in the mean values of TSH, T4, and T3 levels in present study were common. Researchers interested in the study of correlation of TSH, T4, and T3 levels and thyroid disorders usually found that TSH levels in hypothyroidism were more than the normal range [16].

Thyroid peroxidase is a enzyme which responsible for thyroid hormone biosynthesis and the mutation in *TPO* gene leading to thyroid dyshormonogenesis [17]. Also, the loss of TPO activity implicates the inability to iodinate tyrosine residues in (Tg) and to couple these residues to form thyroid hormones, mainly T3 and T4[18]. Total iodide organification defect (TIOD) is autosomal recessive mode and the mutant *TPO* alleles associated with thyroid hormone insufficiency [19].

Thyroid peroxidase is responsible for the synthesis thyroid hormone and catalyzes both iodination and coupling of iodotyrosine in thyroglobulin (Tg) [20]. TPO enzyme is a glycosylated membrane bound hemoprotein localization the apical membrane of the thyrocyte where it plays an essential t role in thyroid hormones synthesis. The exon (7,8 and 9) encoding the catalytic heme binding domain of the TPO enzyme [6]. Al-Deresawi and Alfaisal, [3] recorded two novel frameshift mutation in exon 9 of TPO gene; c.1471delC and c.1481delC leading to insufficient thyroid peroxidase enzyme. Pang and Chan, [17] reported the *TPO* molecular defect leading to the next situations: TPO unable to bind heme molecule, TPO cannot bind with thyroglobulin or iodide (I) as substrate and abnormal TPO lead to wrong cell membrane localization.

CONCLUSIONS:

The down-expression of *TPO*-mRNA caused by the mutations in TPO gene and this mutation leading to defect in thyroid peroxidase configuration and consequencely cause's thyroid dyshormonogenesis.

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