Original Article

Dual Oxidase 1, 2 Gene Expression in Women With Polycystic Ovary Syndrome (PCOS)

Ardeshir Hesampour; Ph.D.[1], Mina Jafarabadi; M.D.[2], Shima Rajabi; M.Sc.[3], Elahe Rezayof; M.Sc.[2], Akram Ghahghaei Nezamabadi; M.D.[4]

1 Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran
2 Vali-E-Asr Reproductive Health Research Center, Family Health Research Institute, Tehran University of Medical Sciences, Tehran, Iran
3 Department of Biology, Central Medical Sciences Unit, Islamic Azad University, Tehran, Iran
4 Department of Obstetrics and Gynecology, Arash Women's Hospital, Tehran University of Medical Sciences, Tehran, Iran

Received August 2023; Revised and accepted November 2023

Abstract

Objective: Dual oxidases (DUOX1, DUOX2) belong to the NADPH oxidase (NOX) family, which produce H2O2 necessary for thyroid hormone biosynthesis. This study aims to evaluate gene expression for DUOX1, DUOX2 in PCOS patients and its relation with thyroid hormone and magnesium levels.

Materials and methods: Totally 88 cases were studied including 24 people with PCOS and hypothyroidism, 44 people with PCOS and normal thyroid function, and 20 hypothyroid patients without PCOS. In comparison 40 healthy controls in the age group of 16-35 years matched for age group and BMI were evaluated. Using Vegaro syringe 5 cc of blood was sampled from all 128 people and after RNA extraction and cDNA synthesis using Real-Time PCR technique, the expression level of DUOX1 and DUOX2 genes was investigated.

Results: The results of hormonal tests showed that there is a significant difference between the level of T4, T3, and TSH hormones in hypothyroid patients with or without PCOS in comparison to the control group. Regarding the level of Mg, the results showed that there is a significant difference between the levels of Mg in PCOS group with or without hypothyroidism in comparison to the control group. Gene expression results showed that the relative changes of DUOX1 gene expression in different groups compared to the control group were significantly reduced $P<0.05$. In the polycystic group with hypothyroidism, the gene expression level showed a decrease compared to the normo-thyroid polycystic group and the hypothyroid non-PCO group, which was statistically significant $P<0.05$.

Conclusion: According to the results of the present study and the previous studies that have been published in the field of Duox1, it can be assumed that the reduction of Duox1 expression can interfere with the oxidative stress system. Further studies with other molecular techniques may help to understand the exact action mechanism of these genes.

Keywords: Dual Oxidase 1 (DUOX1) Gene; Dual Oxidase 2 (DUOX2) Gene; Polycystic Ovary Syndrome; Real-Time Polymerase Chain Reaction (PCR)

Introduction

Polycystic Ovary Syndrome (PCOS) is a heterogeneous endocrine disorder or hormonal imbalance that affects one in 15 women worldwide. A major endocrine disorder in this regard is the

Correspondence:
Dr. Mina Jafarabadi
Email: minajaf@yahoo.com

secretion or overactivity of androgens, and a large proportion of women also have abnormal insulin activity (1).

As mentioned, women with polycystic ovary syndrome have hormonal imbalances that disrupt the menstrual cycle, ovulation, and possibly pregnancy. These hormones are like a complex network, and the function of the female reproductive system depends heavily on its balance (2-4).

Increasing evidence over the years suggests familial accumulation of women with PCOS, hyperandrogenism, and metabolic changes. The PCOS inheritance model has not yet been defined. Some researches link autosomal dominant transmission to a genetic defect (5), but most authors define PCOS as a multi-gene pathology.

A specific gene may also have a dominant effect on a family and affect phenotypic manifestations of the syndrome. The main candidate genes are those that encode factors involved in the synthesis, transfer, regulation and effects of androgens. Other candidate genes are those that are responsible for insulin secretion for factors involved in insulin metabolism, such as insulin receptors, signaling cascade proteins responsible for binding insulin to its receptor, the IGF-1 system, other growth factors, and the Calpain-10 enzyme encoding gene (6, 7).

There is also a relationship between proinflammatory genotypes and PCOS associated with polymorphism of TNF-a and IL-6 receptor encoding genes. Finally, recent evidence of gonadotropin-independent primary folliculogenesis change in women with PCOS suggests that genes involved in folliculogenesis may also play a role in the otopathogenesis of this syndrome (7).

However, only a few PCOS sensitive genes have been repeatedly identified in studies of women of Chinese or European ancestry: allele variants of fibrillin-3 (FBN3), and LH receptor (LHR) variants, FBN3. A protein encodes an extracellular matrix that regulates the signaling of transformative growth factor (TGF). The allele type associated with its PCOS, A8, represents a distinct metabolic phenotype, including insulin resistance.

There are various environmental factors such as diet, physical activity, and overall lifestyle (8).

Magnesium: Magnesium, a mineral abundant in the body, is naturally present in many foods, is added to other food products, is available as a dietary supplement, and is present in certain medications (such as antacids and laxatives). Magnesium is a cofactor in more than 300 enzyme systems that regulate various biochemical reactions in the body, including protein synthesis, muscle and nerve function, blood glucose control, and blood pressure regulation. Magnesium is needed for energy production, oxidative phosphorylation, and glycolysis (9).

It contributes to bone structural growth and is needed for the synthesis of DNA, RNA and the antioxidant glutathione. Magnesium also plays a role in the active transport of calcium and potassium ions in cell membranes, a process that is important for the conduction of nerve impulses, muscle contraction and normal heart rhythms (10).

Usually, low magnesium intake causes changes in biochemical pathways that can increase the risk of disease over time. This section focuses on four diseases and disorders that magnesium may be involved in high blood pressure and cardiovascular disease, type 2 diabetes, osteoporosis, and migraine headaches.

Evidence suggests that magnesium deficiency may play an important role in women's health in several clinical conditions, including premenstrual syndrome, dysmenorrhea, and PCOS. Women with PCOS have lower serum magnesium levels than healthy people. Magnesium can help reduce pain and plays a role in the formation of proteins, cell growth, and cell division involved in hair. Therefore, it is hypothesized that magnesium intake can improve hair loss in women. Magnesium may also have a beneficial effect on skin lesions and acne (11).

Thyroid: The DUOX1 and DUOX2 genes, previously called THOX1 and THOX2, respectively, were first cloned from human and porcarin thyroid gland tissue (12). The DUOX2 gene is located on chromosome 15. It produces an mRNA of 6,532 nucleotides, which encodes a protein composed of 1,548 amino acids. The DUOX1 gene is located at the same location as DUOX2 and encodes a protein of 1551 amino acids that, with DUOX2, has more than 77% sequence similarity at the amino acid level (13).

DUOX1 Gene: The protein encoded by this gene is a glycoprotein and a member of the NADPH oxidase family. Thyroid hormone synthesis is catalyzed by a protein complex located in the apical membrane of thyroid follicular cells. The complex contains a vector of iodide, thyroperoxidase and a peroxide-producing system that contains proteins encoded by this gene and a similar gene to DUOX2 (14).

DUOX1 (two oxidase 1) is a protein encoding gene. Diseases associated with DUOX1 include congenital hypothyroidism and chronic

granulomatous, autosomal recessive disease. Its related pathways include the metabolism of amine-derived hormones and regulation of SLIT and ROBO expression. This gene produces hydrogen peroxide which is necessary for thyroid peroxidase (TPO) and lactoperoxidase (LPO) activity. It plays a role in the synthesis of thyroid hormones and antimicrobial defenses mediated by lactoperoxidase at the mucosal level (15).

DUOX2 Gene: DUOX2 (two oxidase 2) is a gene that encodes the protein. The disease associated with DUOX2 is thyroid Dyshormonogenesis. Among its related pathways are the metabolism of amine-derived hormones, thyroid hormone production and environmental downstream signaling effects.

Hydrogen peroxide is required for thyroid peroxidase (TPO) and lactoperoxidase (LPO) activity. It plays a role in the synthesis of thyroid hormones and antimicrobial defenses mediated by lactoperoxidase at mucosal level (15).

Gene expression: A gene is considered to be the molecular unit of inheritance in all living organisms and may be regarded as the nucleic sequence of acids encoding a functional product such as protein or RNA. If genes produce a protein product, the result of transcription is mRNA, which is the mRNA that triggers the production of the protein. Also, a gene can produce noncoding RNAs that have different functions in the cell, and one of their most important functions is to regulate gene expression.

The process of gene expression in cells comes from several stages including transcription, post-translational changes, translation, and post-translational changes. A summary of the expression of mRNA and miRNA is depicted in the figure. Transcription is the first step in gene expression in which a specific part of DNA is transcribed into RNA by RNA polymerase enzyme in the nucleus. In eukaryotes, transcription begins by binding RNA polymerase to the promoter sequence in DNA in the presence of a variety of transcription factors (TF) (16).

Real-time PCR: (Real-Time PCR) has been developed to investigate the expression of living organisms' genes. The limitation that a simple PCR system has created is the inability to quantitatively examine the expression of a gene. Therefore, a system called real-time PCR (Real-Time PCR) has been developed and developed. What is clear is that the ultimate goal is the genes of living organisms and the entire genome complex is trying to make RNA and protein products.

What can determine what features a cell exhibits at the phenotypic level and at what level it exhibits that feature are the RNA or ribonucleic acids in that cell? Also, to compare the two cells in terms of phenotypic characteristics, they cannot be examined at the DNA level, because DNA is always constant and a specific gene is very similar among organisms of the same species and does not provide any indication of the level of expression of that gene. Therefore, the expression of a gene can be done at the level of RNA or protein.

In a real-time PCR (Real-Time PCR) system to investigate the expression of a gene, the RNA molecules are first transformed into complementary DNA, commonly called cDNA, with the help of reverse transcriptase enzymes. After the stability of the measured molecule, the real-time PCR cycles and the expression is evaluated. Real-time PCR uses an optical system that calculates the DNA of each sample.

When real-time PCR begins its polymerization cycles, for the creation of new DNA strands, the SYBR Green color binds to the strand and the real-time PCR (Real-Time PCR) device starts to read fluorescence by its optical system. Therefore, a real-time PCR device with SYBR Green fluorescence property draws a replication graph for each sample.

Each point of the amplification graph represents the degree to which the SYBR Green binds to the DNA strand, and the graph that is displayed in the real-time PCR (Real-Time PCR) display reaches its threshold. This represents the amount of DNA expressed in each sample (16).

Therefore, considering that the comparisons that are reported quantitatively have a higher value than qualitative reports, the real-time PCR technique is also more valuable than techniques such as RT-PCR, which reports the amount of RNA produced in the cell qualitatively (16).

Considering that hypothyroidism is one of the causes of polycystic ovary syndrome and mutations in two DUOX1 and DUOX2 genes are the main cause of this disease (17-20) and so far its expression of DUOX1 and DUOX2 genes in polycystic ovary syndrome has not been investigated and also in Iran, there has not been any study in this regard, Since magnesium levels can be an important factor in the occurrence of polycystic ovary syndrome, we now measure the effect of magnesium and thyroid hormone (T3, T4, TSH) in patients with polycystic ovary syndrome (PCOS), healthy and the effect of magnesium levels and thyroid hormone (T3, T4,

TSH) in altering the expression of two DUOX1 and DUOX2 genes that cause PCOS.

Materials and methods

Sample Collection: Based on the case-control formula, blood sampling of 5cc syringe and vagaru syringe sampling from 128 patients of which 24 patients with PCOS have hypothyroidism and 44 with normal thyroidism PCOS, and 20 hypothyroidism and 40 healthy controls in the age group of 16-35 years old, all of whom are similar in age and BMI by giving the patient a consent form. It was done to get the blood.

Case-control formula:

$$\alpha = 0.1 \quad \alpha\text{-}1 = Z \quad \beta = 0.20$$

Prevalence ratio of hypothyroidism; p = 0.02

$$S = p(1\text{-}q) = 0.198$$

$$OR = S/T \qquad T = q(1\text{-}p) = 0.0098$$

$$\tau = S+T$$

$$N = \frac{\left[Z1-\frac{\alpha}{2}(OR+1)+Z1-\beta\sqrt{(OR+1)^2}-\sqrt{(OR-1)^2\tau}\right]^2}{(OR-1)^2\tau}$$

(Sample Size 0/1) = 128 116+12= N

5CC blood was taken from each person, part of the blood was sent to the laboratory for separating serum from blood by a desktop centrifuge (model ROTOFIX 32A by HETTICH Germany) and was used to measure the variable (magnesium) by Hitachi device (model 912, Japan). Also, the level of TSH, T3 and T4 of all of these people who had been tested before were examined in their medical history.

Total RNA extraction and isolation: 1000 µl of RNA extraction solution (Trizole of Genix Company) was added to the blood samples. After cell lysis, the solution was transferred to 1.5 ml RNase free tubes and incubated at ambient temperature for 5 minutes.

To the samples, 200 µl of cold chloroform was added and microtube inverted to uniform solution. Microtubes were then placed on an ice vessel for 5 minutes. The samples were centrifuged at a rate of 12000 rpm at 15 min and 4 °C.

After the completion of the detachable three-phase centrifuge, it is observed:

The aqueous phase contains RNA, interphase, DNA and the organic phase where proteins are present. The high transparent phase layer containing Total RNA was carefully drawn and transferred to a new 1.5 ml vial. In order to concentrate RNA and remove the salts in the solution, 700 mL isopropanol alcohol was added and after inverting, it was placed on ice for 15 minutes.

The samples were centrifuged at 12000 RPM for 15 minutes at 4°C. At the end of the vial, RNA deposition was observed. Then the supernatant was discarded and for washing, 1 ml of 75% alcohol was added. Microtubes were inverted several times, then the samples were centrifuged at a rate of 14000RPM for 5 minutes at 4°C.

After the completion of the centrifuge, the supernatant was discharged carefully and for evaporation of alcohol, microtubes were inverted onto sterile gas for 10 minutes. The samples were then placed in a dry block (60°C) for 10 minutes. Then, to protect the samples from RNase and DNAES, the sediment was added to the 30-50 µl DEPC water.

For storing RNA after extraction it should be done in ethanol or isopropanol at -70 because it is most stable at this temperature. Ethanol and isopropanol removed by centrifuge and stored in a buffer without RNase. After extracting RNA, the samples were measured for their quantity and quality, UV-VIS spectrophotometry was performed using nanodrop.

Investigation of RNA Quantity with Nanodrop: To determine the amount of RNA obtained from each sample, Nano-Drop (Bio-RAD550, USA) is used, which determines the RNA concentration of samples at the wavelength of 260nm. Also, to determine the purity of RNA and the presence of DNA contamination, the ratio (A260/A280) of each sample was calculated and the pure RNA sample shows a number between 1.8-2. In addition, the absorption rate of the sample at A260/A230 indicates contamination with phenol or chloroform, which should be about 2-2.2.

In spectrophotometry, the purity of the RNA sample can be investigated using the absorption of light at 260 nm and using the following equation:

Formula

$C\ (\mu g/\mu l) = A260\times\varepsilon\times d/1000$

C: Concentration

A260: Optical absorption at a wavelength of 260 nm

ε: The molar extinction factor is 40 for RNA and 50 for DNA

D: The dilution coefficient

Verification of RNA: Electrophoresis gel with 2% Agarose was used to determine RNA quality and to ensure microRNAs. If we can see the 28s, 18s, and 5s bands of rRNA in the gel as strong (SHARP) bands and smears between them, we can ensure that RNA and miRNAs are present in the extraction product. In this study, the Millennium TM markers

(Thermo Fisher Scientific, InvitrogenTM, catalog number: AM7150) were used.

Method of cDNA synthesis: At first, the concentration of extracted RNA was read by nanodrop, and the OD of each sample was in terms of ng/µl. According to the instructions of the kit, the number of 500 divided by concentration was obtained and finally, the amount of RNA required for synthesis of 20 µl cDNA was obtained and added to the microtubes (Table 1):

Table 1: Materials used for cDNA synthesis

Materials used	Volume (microliter)
Total RNA	500 ng
Buffer-Mix(2X)	10
Enzyme Mix	2
DEPC Treated Water	To volume 20

The materials were quickly vertically modified in microtubes. Then, the reaction mixture was incubated in thermal cyclone according to the cDNA synthesis kit for 10 minutes at 25°C. Then incubated for 60 min at 47°C. By heating for 5 minutes at 85°C (to neutralize reverse transcriptase enzyme), the reaction was stopped. It was immediately placed on ice for 4 minutes. Then, the synthesized cDNA was stored at -20 for further reactions.

Designing a Primer for Real-Time PCR: A primer is a short strand of RNA or DNA, usually consisting of 18 to 22 bases. This short strand serves as a starting point for DNA synthesis. The primer is an essential component in the DNA replication process because the catalyzed enzymes (DNA polymerases) can only add nucleotides to an existing strand of DNA. The polymerase starts the transcription at the end of the 3' primer and starts copying the front string. DNA primers have higher temperature resistance.

In order to design primers by this method, mRNA sequence was extracted from http://www.NCBI.org site, then primer 3 plus was designed for the target genes and finally BLAST sites were specially approved and GAPDH internal control gene was considered. The sequence of designed primers is shown in the table 2.

Requirements for Real-Time PCR: 2X Real-Time PCR Master Mix kit

Water treated with DEPC, Sinaclan Company (Iran)

Primer: For PCR RT-reactions ،cDNA was used as template with specific primers designed for genes. It was used for internal control of the GAPDH gene (Tables 3 and 4).

Statistical analysis: The statistical calculations of this study were performed using GraphPad software and the data were analyzed by one-way ANOVA, and the difference between target gene expression and between different groups was calculated using Tukay's multiple comparison test.

Table 2: The sequence of designed primers

Name of the gene	(5'-3') Primer sequence	Duplicate fragment (open pair)
DUOX1	TCTGGTCCTGCCCTCTTACA GCCTCCAGTACAGTGTCATTGC	114 bp
Buffer-Mix(2X)	GGTCACGCAGTCAATGTCTAC CCTGGGACGGTCTGGAAG	137 bp
DUOX2	CATCAAGAAGGTGGTGAAGCAG GCGTCAAAGGTGGAGGAGTG	120 bp

The difference between the mean of the groups for all tests was significant at >0.05 level.

Table 3: Materials used for gene replication

Materials	Volume (microliter)
SYBR Green Master Mix	7.5
PCR forward primer (3pM)	0.5
PCR reverse primer (3pM)	0.5
Template (<100ng)	1
dH_2O	5.5
Total	15

One of the methods by which SYBR-Green Real-time PCR data is analyzed is the relative evaluation method. In this method, the expression of a target gene is compared with the expression of the housekeeper gene. Different methods can be used to evaluate the relative expression of these genes. The method used in this study includes ΔΔCt method based on a direct comparison of CTs. First, the ΔCt of each sample is calculated by determining the difference between the target gene CT and the internal control gene CT level.

Table 4: The real time PCR schedule for gene replication

Steps	Temperature (°C)	Time	Cycle
Holding	95	10 min	1
Denaturation	95	15 sec	40
Annealing	50-60	12 min	35-40
Extension	72	30 sec	35-40

Melt Analysis

This calculation should be performed for all target samples and calibrator samples (control or control).

$$\Delta CT \text{ (sample)} = CT \text{ target gene} - CT \text{ reference gene}$$

$$\Delta CT \text{ (calibrator)} = CT \text{ target gene} - CT \text{ reference gene}$$

In the next step, the ΔΔCT level of each sample is obtained by subtracting the ΔCT calibrator from the target sample.

$$\Delta\Delta CT = \Delta CT \text{ (sample)} - \Delta CT \text{ (calibrator)}$$

If the amplification efficiency of the target gene and internal control gene is comparable, the normalized level of target gene expression is calculated using the following formula:

Normalized target gene expression level in sample = $2^{-\Delta\Delta CT}$

However, if the efficiency of the target gene amplification and the internal control gene are not comparable, this method of calculation may lead to an incorrect determination of the level of expression of the target gene. This error is related to the performance efficiency and the number of real-time PCR cycles but can be calculated in the following way:

$$\text{Error (\%)} = [(2n/(1 + E)n) \times 100)] - 100$$

(E = efficiency of PCR; n = cycle number)

Results

The distribution of cases according to age groups is summarized in table 5.

Table 5: Age groups of participants in PCO and control participants

Age groups (years)	PCO	Control	P value
15-20	4	1	0.68
20-30	31	12	
30-40	53	27	

Distribution of T4 serum level in studied groups is shown in Figure 1 and table 6.

The level of T4 hormone in hypothyroid patients and polycystic patients with hypothyroidism is lower than in pure polycystic patients and healthy individuals.

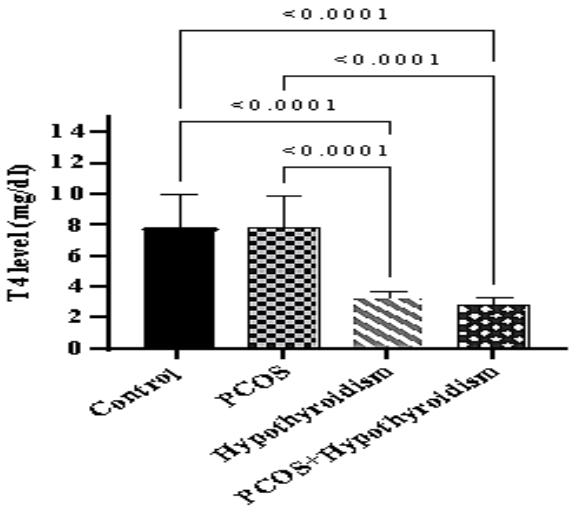

Figure 1: Distribution of T4 serum level in studied groups

The distribution of T3 serum level in studied groups is shown in Figure 2 and table 7.

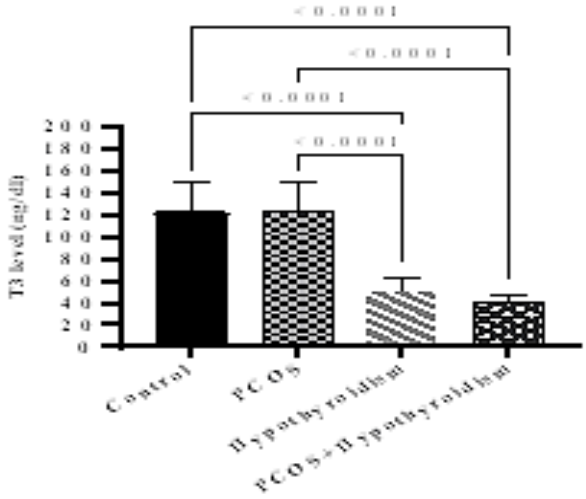

Figure 2: Distribution of T3 serum level in studied groups

Table 6: Tukey's multiple comparisons test for serum level of T4

Perinatal Outcome	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Control vs. PCOS	0.1855	-0.7484 to 1.119	0.9548
Control vs. Hypothyroidism	4.630	3.459 to 5.801	<0.0001
Control vs. PCOS+Hypothyroidism	5.044	3.940 to 6.148	<0.0001

PCOS vs. Hypothyroidism	4.445	3.292 to 5.597	<0.0001
PCOS vs. PCOS+Hypothyroidism	4.859	3.774 to 5.943	<0.0001
Hypothyroidism vs. PCOS+Hypothyroidism	0.4142	-0.8800 to 1.708	0.8385

Table 7: Tukey's multiple comparisons test for serum level of T3

	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Control vs. PCOS	-3.795	-16.30 to 8.709	0.8587
Control vs. Hypothyroidism	71.75	56.07 to 87.43	<0.0001
Control vs. PCOS+Hypothyroidism	80.68	65.90 to 95.46	<0.0001
PCOS vs. Hypothyroidism	75.55	60.11 to 90.98	<0.0001
PCOS vs. PCOS+Hypothyroidism	84.48	69.95 to 99.00	<0.0001
Hypothyroidism vs. PCOS+Hypothyroidism	8.933	-8.396 to 26.26	0.5378

The level of T3 hormone in hypothyroidism patients and polycystic patients with hypothyroidism is lower than in pure polycystic patients and healthy people.

The distribution of TSH serum levels in studied groups is shown in figure 3 and table 8.

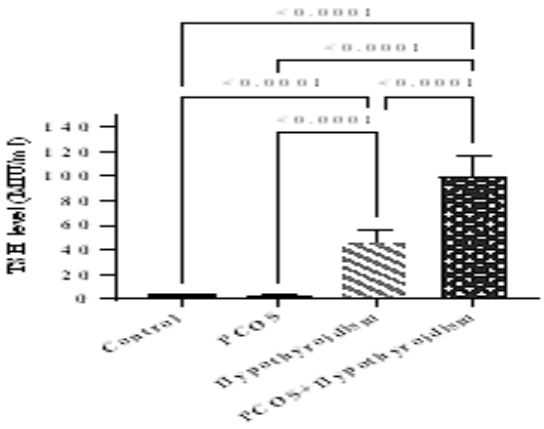

Figure 3: Distribution of TSH serum level in studied groups

The level of TSH hormone in hypothyroid patients and polycystic patients with hypothyroidism is more than polycystic pure patients and healthy individuals.

Distribution of Mg serum level in studied groups is shown in figure 4 and table 9.

In a diagram, Heatmap means a heat map, which displays complex data sets as images of different colors to make them easier to understand. Figure 5 is a chart that clearly shows a significant difference between the levels of Mg and thyroid hormones (TSH, T3, T4) in polycystic patients and polycystic patients with hypothyroidism and hypothyroidism and control.

The relative expression of DUOX1 gene in different groups after RNA extraction and cDNA synthesis from blood samples was measured by time PCR Real technique and GAPDH primer as internal control.

The results showed that relative changes in DUOX1 gene expression in different groups were significantly reduced compared to the control group P <0.05. In the polycystic group with hypothyroidism, gene expression decreased more than polycystic group and hypothyroidism group, which was statistically significant P<0.05 (Figure 6).

This study can conclude that decreased DUOX1 expression can be associated with thyroid hormones, causing polycystic ovary syndrome (Table 10).

DUOX1 and GAPDH gene replication and melting chart (figures 7 & 8)

In the graph of gene replication, there are three stages, the Log phase, which is the stillness phase that has not yet begun to proliferate, the Exponential phase in which the gene is reproduced logarithmically, and the stationary phase in which PCR materials are finished and no longer proliferated. This graph is based on fluorescent and cyclic intensity. In a particular cycle, the gene begins to multiply.

To further verify that only the product has been reproduced and the primer has been specifically performed and the product has not been duplicated incorrectly, the Real Time PCR product was taken on 1% agarose gel.

Table 8: Tukey's multiple comparisons test for serum level of TSH

	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Control vs. PCOS	0.3427	-4.401 to 5.087	0.9976
Control vs. Hypothyroidism	-42.28	-48.22 to -36.33	<0.0001
Control vs. PCOS+Hypothyroidism	-96.34	-101.9 to -90.73	<0.0001

PCOS vs. Hypothyroidism	-42.62	-48.47 to -36.76	<0.0001
PCOS vs. PCOS+Hypothyroidism	-96.68	-102.2 to -91.17	<0.0001
Hypothyroidism vs. PCOS+Hypothyroidism	-54.07	-60.64 to -47.49	<0.0001

Table 9: Tukey's multiple comparisons test for serum level of Mg

	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Control vs. PCOS	0.7002	0.5331 to 0.8672	<0.0001
Control vs. Hypothyroidism	0.02125	-0.1881 to 0.2306	0.9935
Control vs. PCOS+Hypothyroidism	0.9059	0.7085 to 1.103	<0.0001
PCOS vs. Hypothyroidism	-0.6789	-0.8851 to -0.4727	<0.0001
PCOS vs. PCOS+Hypothyroidism	0.2058	0.01175 to 0.3998	0.0331
Hypothyroidism vs. PCOS+Hypothyroidism	0.8847	0.6532 to 1.116	<0.0001

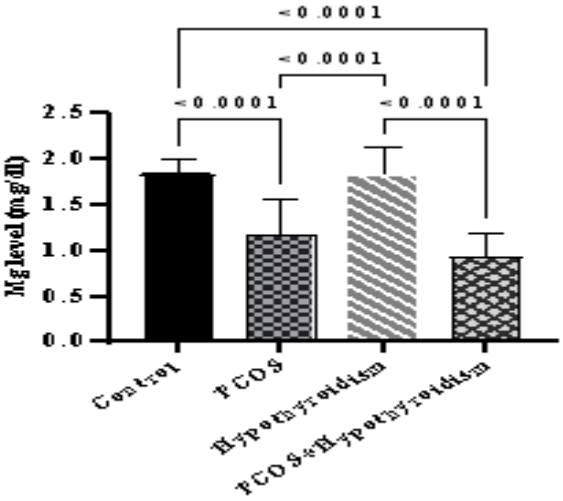

Figure 4: Distribution of Mg serum level in studied groups

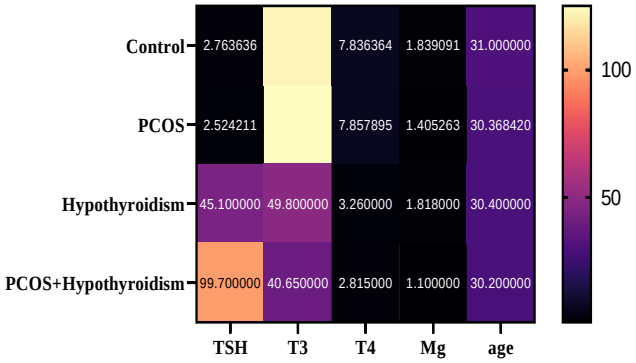

Figure 5: the levels of Mg and thyroid hormones in studied groups

The product length of the DUOX1 gene is 114 nucleotides (Figure 9) and the GAPDH gene product length is 120 nucleotides (Figure 10).

Discussion

The results of the hormonal assay showed a significant difference between the levels of T4, T3, and TSH in hypothyroidism and polycystic patients with hypothyroidism compared to the control group. Also, there is a significant difference between the levels of T4, T3, and TSH in hypothyroid patients compared to polycystic patients and there is also a significant difference between the level of T4 hormone in polycystic patients compared to polycystic patients with hypothyroidism.

The results showed that there was a significant difference between the levels of Mg in polycystic and polycystic patients with hypothyroidism compared to the control group.

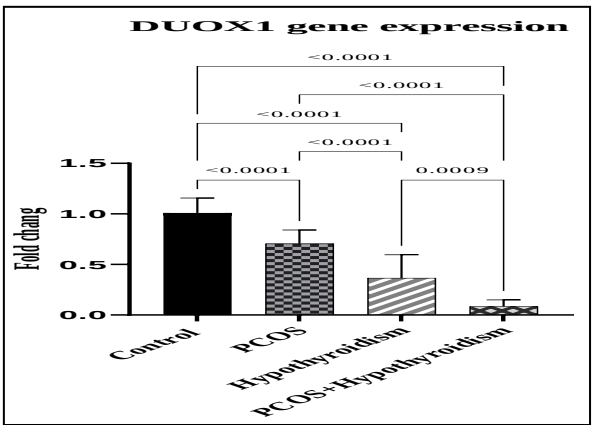

Figure 6: Results of DUOX1 gene expression

Table 10: Tukey's multiple comparisons test for DUOX1 expression

	Mean Diff.	95.00% CI of diff.	Adjusted P Value
Control vs. PCOS	0.3045	0.1507 to 0.4582	<0.0001
Control vs. Hypothyroidism	0.6442	0.4669 to 0.8215	<0.0001
Control vs. PCOS+Hypothyroidism	0.925	0.7477 to 1.102	<0.0001

PCOS vs. Hypothyroidism	0.3397	0.1812 to 0.4982	<0.0001
PCOS vs. PCOS+Hypothyroidism	0.6205	0.4620 to 0.7791	<0.0001
Hypothyroidism vs. PCOS+Hypothyroidism	0.2808	0.09934 to 0.4623	0.0009

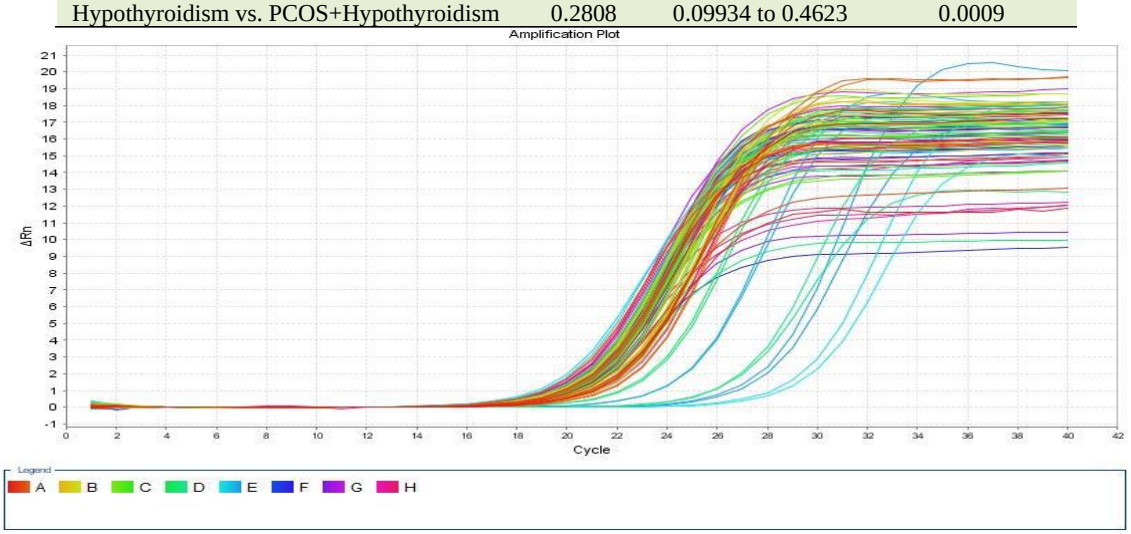

Figure 7: GAPDH gene duplication diagram

Also, there is a significant difference between the level of Mg level in hypothyroidism patients compared to polycystic patients and there is also a significant difference between the levels of Mg level in hypothyroidism patients compared to polycystic patients and polycystic patients compared to hypothyroidism patients.

Also, the results showed that relative changes in DUOX1 gene expression in different groups compared to the control group had a significant reduction of $P<0.05$. In the polycystic group with hypothyroidism, gene expression decreased more than polycystic group and hypothyroidism group, which was statistically significant $P<0.05$.

Previous studies have shown magnesium to play a role in most important physical functions such as thyroid function (17). In fact, this mineral is necessary for the secretion of thyroid hormones. Magnesium deficiency has been known to be one of the contributing factors in polycystic ovary syndrome (18). The present study shows that the level of Mg in polycystic patients and polycystic patients with hypothyroidism has decreased significantly compared to healthy individuals and hypothyroidism patients.

Also, decreased TSH levels are more common in polycystic patients with hypothyroidism than polycystic patients and healthy individuals which is in concordance with other studies (19).

In this study, DUOX2 was not expressed in the blood**.** In order to understand the exact mechanism of these genes, more studies with more molecular techniques must be done.

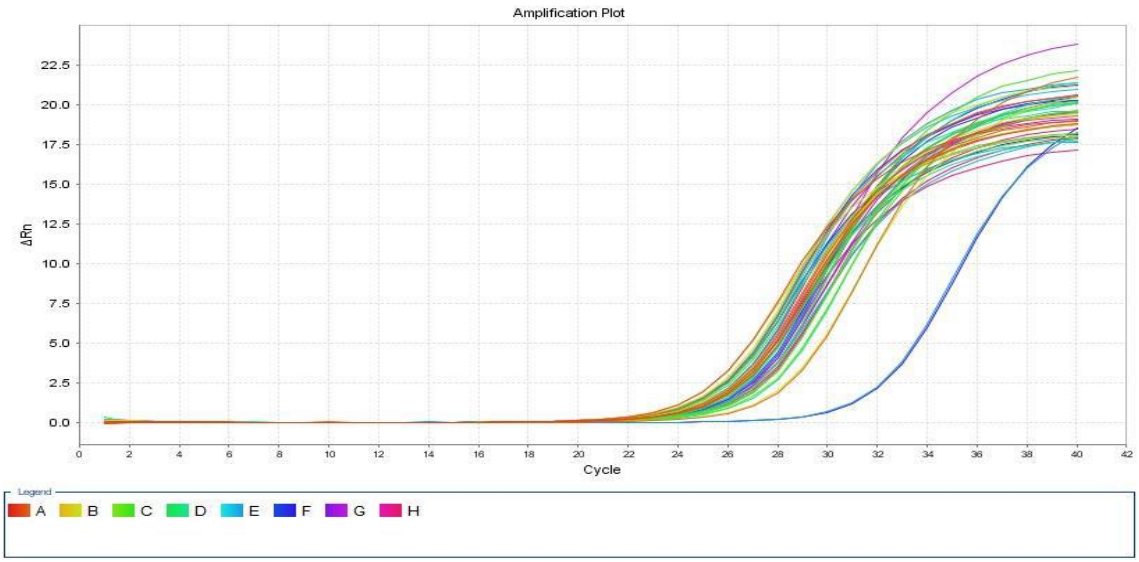

Figure 8: DUOX1 gene duplication diagram in different groups

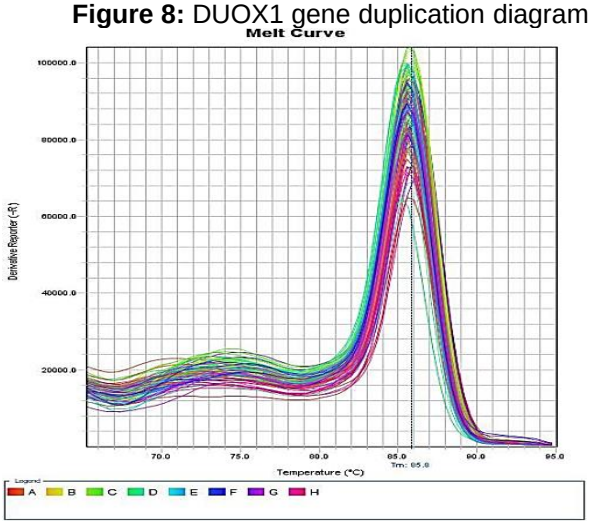

Figure 9: Melting chart of DUOX1 gene, PCR product has 43.85: Tm

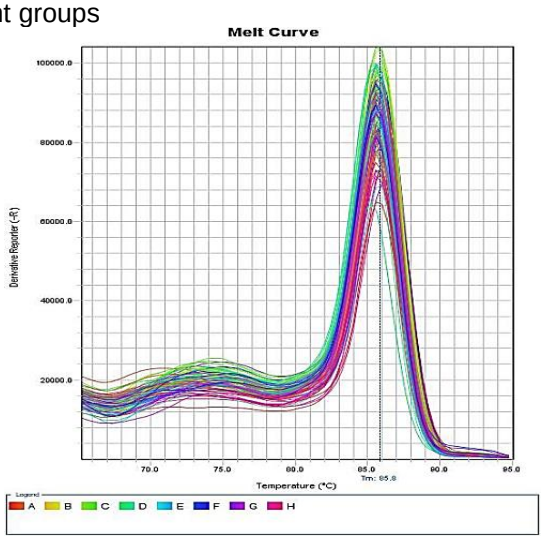

Figure 10: GAPDH gene melting diagram, PCR product has 8.85: TM

It is suggested to use other tissues and compare between groups, to use other techniques such as Western blot, and to use the genes of other pathways in future studies.

Conclusion

According to the results of the present study and previous studies published in the field of DUOX2 and DUOX1, it can be assumed that a decrease in DUOX1 expression can interfere with the relationship with thyroid hormones and magnesium levels and cause polycystic ovary syndrome.

Conflict of Interests

Authors declare no conflict of interests.

Acknowledgments

None.

References

1. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. Lancet 2007;370(9588):685-97.
2. Grassi G, Polledri E, Fustinoni S, Chiodini I, Ceriotti F, D'Agostino S, et al. Hyperandrogenism by Liquid Chromatography Tandem Mass Spectrometry in PCOS: Focus on Testosterone and Androstenedione. J Clin Med 2020;10(1):119.
3. Cho LW, Jayagopal V, Kilpatrick ES, Holding S, Atkin SL. The LH/FSH ratio has little use in diagnosing polycystic ovarian syndrome. Ann Clin Biochem 2006;43(Pt 3):217-9.
4. Caglar GS, Kahyaoglu I, Pabuccu R, Demirtas S, Seker R. Anti-Mullerian hormone and insulin resistance in classic phenotype lean PCOS. Arch Gynecol Obstet 2013;288(4):905-10.
5. Hart R, Hickey M, Franks S. Definitions, prevalence and symptoms of polycystic ovaries and polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2004;18(5):671-83.
6. Franks S, McCarthy M. Genetics of ovarian disorders: polycystic ovary syndrome. Rev Endocr Metab Disord 2004;5(1):69-76.
7. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. Reprod Biol Endocrinol 2016;14(1):38.
8. Escobar-Morreale HF, Luque-Ramírez M, San Millán JL. The molecular-genetic basis of functional hyperandrogenism and the polycystic ovary syndrome. Endocrine reviews 2005;26(2):251-82.
9. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington (DC): National Academies Press (US); 1997.
10. Gröber U, Schmidt J, Kisters K. Magnesium in Prevention and Therapy. Nutrients 2015;7(9):8199-226.
11. Hamilton KP, Zelig R, Parker AR, Haggag A. Insulin Resistance and Serum Magnesium Concentrations among Women with Polycystic Ovary Syndrome. Curr Dev Nutr 2019;3(11):nzz108.
12. Sarr D, Tóth E, Gingerich A, Rada B. Antimicrobial actions of dual oxidases and lactoperoxidase. J Microbiol 2018;56(6):373-386.
13. Faria CC, Fortunato RS. The role of dual oxidases in physiology and cancer. Genet Mol Biol 2020; 43(1 suppl. 1):e20190096.
14. Jing L, Zhang Q. Intrathyroidal feedforward and feedback network regulating thyroid hormone synthesis and secretion. Front Endocrinol (Lausanne) 2022;13:992883.
15. Rigutto S, Hoste C, Grasberger H, Milenkovic M, Communi D, Dumont JE, et al. Activation of dual oxidases Duox1 and Duox2: differential regulation mediated by camp-dependent protein kinase and protein kinase C-dependent phosphorylation. J Biol Chem 2009;284(11):6725-34.
16. Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet 2011;12(2):99-110.
17. Kolanu BR, Vadakedath S, Boddula V, Kandi V. Activities of Serum Magnesium and Thyroid Hormones in Pre-, Peri-, and Post-menopausal Women. Cureus 2020;12(1):e6554.
18. Farsinejad-Marj M, Azadbakht L, Mardanian F, Saneei P, Esmaillzadeh A. Clinical and Metabolic Responses to Magnesium Supplementation in Women with Polycystic Ovary Syndrome. Biol Trace Elem Res 2020;196(2):349-358.
19. Singla R, Gupta Y, Khemani M, Aggarwal S. Thyroid disorders and polycystic ovary syndrome: An emerging relationship. Indian J Endocrinol Metab 2015;19(1): 25-9.

Citation: Hesampour A, Jafarabadi M, Rajabi S, Rezayof E, Ghahghaei Nezamabadi A. **Dual oxidase 1, 2 Gene Expression in Women With Polycystic Ovary Syndrome (PCOS).** J Family Reprod Health 2023; 17(4): 205-15.