Detection of five substitution TPO mutations in Polycystic Ovary Syndrome (PCOS) and thyroid hormones disturbance patients

تحديد خمسة طفرات TPO إستبدالية في مرضى متلازمة تعددالاكياس المبيضية واختلال هرمونات الدرقية

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Abstract

Polycystic ovary syndrome (PCOS) is a complex disorder which reflects variable clinical symptoms. There is considerable heterogeneity of symptoms and signs among women with PCOS, and for an individual, these may change over time. The genetic base of PCOS is not clear and no concrete genetic correlation was built with PCOS. The current study showed the presence of multiple peripheral small cysts 5-9 mm also there was an increase in ovarian volume 3.7-3.9 cm and change in ovarian dimensions with the ovary being more spherical. 20.8% of the PCOS patients included in this study found to have hyperthyroidism. Thethyroid stimulating hormone-TSH level was significantly higher 17.34±5.12µIU/ml in 4 POCS patients with thyroid hormones disturbance than the level in POCS patients without thyroid disturbance or healthy control group (2.19 ±0.47 and 2.33±0.44 µIU/ml respectively). On the other hand, the rest of POCS patients with thyroid hormones disturbance 7 Patients showed lower significance levels of triiodothyronine-T3 and thyroxine-T4 (0.96±0.029 and 51.33± 10.96 n.mol/L respectively) than other groups. At the molecular analysis five substitution thyroid peroxidase genes -TPO mutations were detected in 5 patients with PCOS and thyroid hormones disturbances. Three of them were detected in exon 8 and two in exon 9. The missense substation mutations detected in this study involve one transition of T to C (c.904T>C) and 4 transversion of C to G, C to A and G to C (c.904T>C, c.1280C>G, c.1265C>A, c.1617G>C and c.1603G>C respectively).

Key wards: PCOS, Thyroid disorders, TPO, Mutations, Hormonal disturbances

المستخلص

متلازمة تعدد الاكياس المبيضية خلل معقد له إنعكاسات واعراض طبية متنوعة وهناك إختلاف واضح في هذه الاعراض والعلامات بين النساءالمصابات بهذه المتلازمة يمتد حتى على مستوى الفرد اذ تتغير هذه الاعراض مع مرورالوقت . الاساس الوراثي لمتلازمة تعددالاكياس المبيضة غيرواضح ولم تبنى أيةعلاقة وراثية مع هذه المتلازمة . توضح الدراسة الحالية وجود أكياس صغيرة متعددة محيطي 5 -9 ملم وزيادة من حجم المبايض3.7 -3.9 سم وتغيرات في أبعاد المبايض بحيث تصبح أكثر كروية . أن 20.8% من مرضى متلازمة تعدد الاكياس المبيضية المشمولين بالدراسة لديهم تضخم في الغدة الدرقية وكان مستوى هرمون TSH مرتفع معنوياً 17.34±5.12 مايكروووحدة دولية/مل في أربعة مرضى من هولاء اكثرمما لدى المصابات بالمتلازمة دون خلل في الهرمونات الدرقية أومجموعةالسيطرة 2.19 ±0.47، 2.33±0.44 مايكروووحدة دولية/مل على التوالي. في مقابل ذلك فأن ماتبقى من المرضى المصابات بالمتلازمة وإختلال هرمونات الدرقية 7 مرضى اظهرن انخفاض معنوي في مستوىT4,T3 0.29 ±0.96 و 51.33 ± 10.96 نانومول/ لترعلى التوالي مقارنة ببقيةالمجاميع . أماعلى المستوى الجزيئي تم تحديد خمسة طفرات TPO إستبدالية في خمسة مرضى من المصابات بالمتلازمة وإختلال هرمونات الدرقية . ثلاثة من هذه الطفرات سجلت في المحورالثامن وأثنان في المحورالتاسع للجين . أشتملت طفرات الاستبدال هذه على استبدال مكافيء واحدلثايمين بالسايتوسين (c.904T>C) وأربعة طفرات أستبدال غيرمكافيء للسايتوسين بالجوانين أو السايتوسين بالادنين أوالجوانين بالسايتوسين C.904T>C, c.1280>G, c.1265C>A, c.1617G>C) و c.1603G>C على التوالي) .

الكلمات المفتاحية: متلازمة تعدد الاكياس المبيضية ، اضطرابات الغدة الدرقية ، الطفرات ، الاضطرابات الهرمونية

Introduction

Polycystic ovary syndrome (PCOS or PCO) is a complex condition that affects the ovaries. In PCOS, the outer surface of the ovary has an abnormally large number of small follicles usually more than 10 or

15 in each ovary and almost none in the middle of the ovary which make the ovaries bigger than average. These follicles are all small and immature and generally do not exceed 10mm in size [1]. This will reduce the fertility of PCOS women and make the ovulation rare with irregular period. PCOS affects women between the ages of 15 and 50 and common among women with ovulation problems [2].The genetic base of the PCOS is not clear and the genetic studies have not as yet concluded the pattern of heredity [3]. However, studies on some cases of PCOS revealed that PCOS abnormal follicular apparatus may be due to X chromosomal factors [4] and a large deletion of the long arm of chromosome 11 was seen in some of the PCOS cases [5]. Other studies were linked the polymorphism in the CYP1A1coding for P450 cholesterol side chain cleavage, CYP17 and CYP21 genes, encoding a steroidogenic enzymes with PCOS susceptibility [6].Clinical studies showed that there is a kind of correlation between PCOS and thyroid disorders [7]. Researchers have shown that women with PCOS are three times as likely to also have an autoimmune thyroid disease such as Grave's disease and Hashimoto's thyroiditis where the thyroid gland cells were attacked by anti-thyroid peroxidase or thyroglobulin antibodies[8]. Other studies were concluded that some thyroid disorders leads to low level of sex hormone binding globulin (SHBG) which increase the testosterone level and producing PCOS symptoms such as infertility, polycystic ovaries, hirsutism, male pattern hair loss and acne [9].Thyroid hormone synergize with FSH to exert direct stimulatory effectson granulose cells function, including morphological differentiation, thyroidhormones facilitate FSH-mediated LH/hCG receptor induction andprogesterone secretion[9,10]. Hence, the occurrence of gonadal dysfunction mayfurther results from inadequate thyroid hormones availability at the level of the ovary [11]. The inadequate thyroid hormones availability due to defect of synthesis of these hormones, the majority of these defects are due to mutations in thyroglobulin-TG and thyroid peroxidase-TPO genes [12]. Mutations among TPO and TG genes associated with thyroid disorders were strongly proved [13,14,15]. The current study is attempted to find the TPO mutations associated with PCOS combined with thyroid hormones disturbances.

Materials and Methods

Patients and Healthy groups:

This study included 11 infertile women with PCOS plus thyroid hormones disturbance, 41 infertile PCOS women with normal thyroid hormones levels and 20 healthy women. The subjects were aged between 25 to 49 years. Samples were collected from Infertility Center of Al-Yarmouk Teaching Hospital from November 2010 to May 2011.

Included Criteria:

POCS patients were chosen according to the presence of polycystic ovary morphology and oligo or anovulation [16] while healthy women were chosen according to normal ultrasonography, regular menstrual cycle and normal endocrinal hormones.

Blood sampling:

Five milliliters from blood was collected from each subjects. 3 ml of each blood sample was used to obtain serum samples. The rest 2 ml blood samples were stored in EDTA tubes for DNA extraction.

Thyroid Hormones assay:

VIDAS T3, T4 and TSH kits (BioMerieux-France) and the VIDAS instrument (Addendum-Mini VIDAS apparatus-VIDAS 12 mode l0, 1992, BioMerieux Company) were used for the quantitative measurement of T3, T4 and TSH serum using the enzyme linked fluorescent assay (ELFA) technique.

Genomic DNA isolation:

The genomic DNA isolated from the blood collected in EDTA anticoagulant using BioDelta DNA extraction kit.

TPO gene Primers:

Two specific designed primers for the exons 8 and 9 of the TPO gene were used in PCR reaction at work solution concentration (10µM).The sequences of these primers were listed in Table (1) .

Table (1): TPO exons 8 and 9 primers sequences.

Exons	Forward	Reverse	size-bp
Exon8	5-act-ccc-ctt-tgc-ctg-cag-ctc-cc-<g>-3	3-ctg-cac-agg-agc-tca-cga-tga-ccc-<t>-5	756
Exon9	5-tgc-ttt-tcc-tat-ctg-cac-aga-tca-tcacc-<c>-3	3-acc-agc-tgc-agg-gac-cgc-act-c-<a>-5	307

PCR Programs:

DNA samples were subjected to PCR using master mix (Promega Corp., Madison, WI), primers listed in Table 1, and a thermal cycle (Applied Biosystem-USA). The standard cycle procedure was a 5-minute denaturation at 95°C for one cycle, then 35 cycles of 45 seconds of denaturation at 95°C, 45 seconds of annealing between 63 to 65°C, 60 seconds extension at 72°C and 7 min for final extension at 72°C.The PCR products 27 samples of the analyzed TPO gene regions and primers were sent to Source BioScience Company (Nottingham, UK) for sequencing.

Results and Discussion

Ultrasound findings

Ultrasound examination of the ovaries showed the presence of multiple peripheral small cysts 5-9 mm, also there was an increase in ovarian volume 3.7-3.9cm and change in ovarian dimensions with the ovary being more spherical. PCOS and hypothyroid women commonly suffer from menstrual irregularities and impaired fertility attributed to an ovulation and/or luteal phase defect. The current results showed that the women with PCOS (with or without hypothyroidism) had significantly larger ovaries when compared with controls, suggesting that PCOS and thyroid dysfunction have a profound effect on ovarian size, and may also produce ovarian cysts. These results were in agreement with many previous studies [17,18], that confirm this fact. The large ovarian volume of the PCOS women can be explained by the presence of many follicles. [19] suggested the increased androgen secretion by the theca cell that may increase the small number of potentially recruit follicles in ovaries, these follicles with the hyperactivity of granulosa cells could cause the an ovulation in PCOS women.

POCS TSH, T4 and T3 profile

Abnormal levels of thyroid hormones were detected in 11 (20.8%) of patients with POCS Table (2). The results revealed that TSH level was significantly higher (17.34±5.12)µIU/ml in 4 POCS patients with thyroid hormones disturbance than the level in POCS patients without thyroid disturbance or healthy control group (2.19 ±0.47 and 2.33±0.44µIU/ml respectively). On the other hand, the rest of POCS patients with thyroid hormones disturbance 7 Patients showed a lower significant levels of T3 and T4 (0.96±0.029 and 51.33± 10.96 n.mol/L respectively) than other groups.

Table (2): Serum Thyroid Stimulating Hormone-TSH, Triiodothyronine- T3, and Thyroxin- T4 in Polycystic Ovary Syndrome women and healthy control group.

Hormonal profile	Patients and Healthy groups		
	PCOS with abnormal hormones levels and with mutations	PCOS with normal hormones levels	Healthy control group
		Mean ± SE	
TSH µIU/ml	↑ 14.8±3.017*	2.33 ± 0.44 ns	2.15 ± 0.50
T3 n.mol/L	↓ 0.87±0.12*	1.81 ± 0.14 ns	1.97 ± 0.16
T4 n.mol/L	↓ 88.6±4.046*	97.10 ± 4.88 ns	96.25 ± 5.13

*** (P<0.05), ns: non-significant.**

The results of PCOS women showed that there was no type of thyroid disorder but hypothyroidism. The results showed that there was only 20.8% of PCOS women were incidence in hypothyroidism. These results were higher than those found by [20] who reported 0.7% of PCOS women with hypothyroidism. Others were reported with the autoimmune thyroid disease (AITD) which was more frequently present in women with endometriosis and PCOS [8,9]. Ovarian cyst formation in women with primary hypothyroidism and in experimentally hypothyroid animal exposed to hyperstimulation with gonadoteopins were also reported [21]. Thyroid hormones are involved in the gonadoteopins induced E2 and progesterone secretion by human granolsa cells [21]. Thyroid hormones synergism with FSH to exert direct stimulatory effects on granulosa cell functions including ovarian morphology, and facilitate FSH-mediated LH/hCG receptor induction and progesterone secretion [11] which means that gonadal dysfunction may further result from inadequate thyroid hormone availability in ovary. Hypothyroidism results in an altered peripheral estrogen metabolism. Decreases in SHBG and its binding activity, together with an altered peripheral metabolism of estrogen may result in abnormal feedback at the pituitary level [22].

TOP mutations profile:

Five substitution TPO mutations were detected in 5 patients with PCOS and thyroid hormones disturbances Table (3) Figure (1,2). All mutations were missense mutations. Three of them were detected in exon 8 and two in exon 9. The missense substation mutations detected in this study involve one transition of T to C (c.904T>C) and 4transversion of C to G, C to A and G to C (c.904T>C, c.1280C>G, c.1265C>A, c.1617G>C and c.1603G>C respectively).

Table (3): TPO mutations detected in PCOS patients with or without thyroid hormones disturbances.

Exon	Type of Mutation	Number of patients with mutations	
		PCOS with abnormal hormones levels	PCOS with normal hormones levels
8 0	c.904T>C Leu274Pro, L274P	1	
0	c.1280C>G Val400Ala, V400A	1	
0	c.1265C>A Asp394Glu, D394E	1	
9 0	c.1617G>C Glu510Asp, E510D	1	
0	c.1603G>C Ala506Pro, A506P	1	

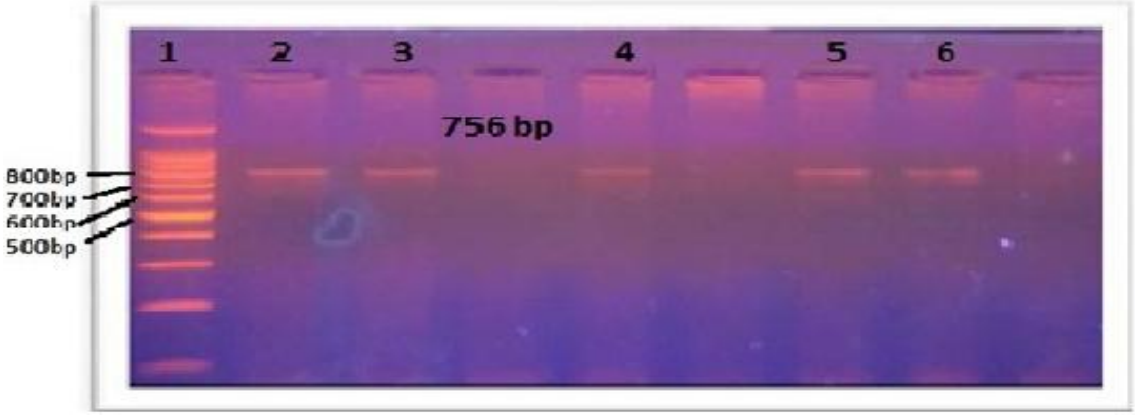

Figure (1): Screening for DNA samples of patients and control for exon 8 of TPO gene. Line 1: Marker, 2: PCR product of healthy sample, 3&4: PCR product of PCOS patients, 5&6: PCR product of PCOS patients with hypothyroidism. Ethedium bromide stained 2% agarose gel electrophoresis carried out for 45 mins at 100 volts.

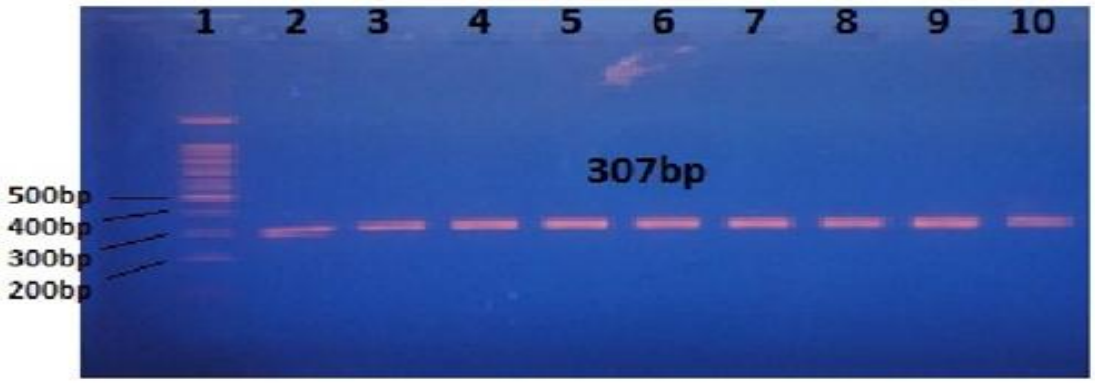

Figure (1): Screening for DNA samples of patients and control for exon 9 of TPO gene. Line 1: Marker, 2&4: PCR product of PCOS patients, 5to8: PCR product of PCOS patients with hypothyroidism, 9&10: PCR product of healthy. Ethedium bromide stained 2% agarose gel electrophoresis carried out for 45 mins at 100 volts.

Hypothyroidism is associated with a broad spectrum of reproductive disorders ranging from abnormal sexual development to menstrual irregularities and infertility. It is commonly associated with failure of ovulation and numerous interactions of thyroid hormones with the female reproductive system were found to have effects on menstrual function and ovulation. PCOS is the most common endocrine disorder in women of reproductive age, affecting about 5 to 10% of all premenopausal women [23]. Polygenic pattern of inheritance in PCOS patients was seen in twin and familial studies [5,24,25]. Several genes were implicated in the pathogenesis of PCOS [5] and complexity of PCOS can be explained by the interaction of a number of key genes responsible for most of the endocrine and metabolic symptoms of the PCOS. Although, the hypothyroidism is known to produce a reversible phenotype similar to that of PCOS, there are no reports of association of hyperthyroidism with PCOS [26].

In the current study, PCOS with hypothyroidism women have shown to have significantly increased TSH and decreased T3 and T4 levels associated with mutations in TPO gene. Our results have revealed that the most detected mutations have promoted effect among synthesis of thyroid hormones. Defect of synthesis the thyroid hormones are common cause of thyroid disorders, the majority of these disorders are due to mutations in TG and TPO genes causing a biosynthesis defect [27]. TPO mutations were also detected in patients with PCOS combined with thyroid disorders [3,27,28]. Missense mutation which a single codon is altered so that one amino acid in protein is replaced with a different amino acid, the severity of a missense mutation depends on the nature and location of the amino acid was substituted. Thyroid peroxidase is a glycosylated membrane bound hemoprotein localized in the apical membrane of the thyrocytes where it plays an essential role in thyroid hormone synthesis. However, [29] reported the TPO molecular abnormalities leading to TPO unable to bind heme; TPO cannot bind with thyroglobulin or iodide as substrate and abnormal TPO lead to wrong cellular localization.

In conclusion, to the best of our knowledge the first attempt to correlate the TPO mutations and thyroid hormones level to PCOS. However, we need to expand this study to provide more cement evidence about this correlation.

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