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Association of Glutathione-S-Transferase Omega 2 (GSTO2) Gene Polymorphism with Incidence of Polycystic Ovary Syndrome in Iraqi Women

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Abstract: Polycystic ovary syndrome (PCOS) is the most frequent endocrinological disorder, occurring in young women. The goal of this study was to look at a possible link between GSTO2 N142D (rs156697) polymorphisms and PCOS, investigating the effect of these polymorphisms on the levels of Luteinizing hormone (LH), Follicle stimulating hormone (FSH), and Testosterone. This study was carried out in the Laboratories of the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies - at the University of Baghdad during the period from 1 November 2021 until the end of June 2022, The PCOS patients were taken from the Kamal Al-Samarrai infertility treatment Hospital in Baghdad. Women with PCOS (n=50) and apparently healthy subject (n=50), were enrolled and GSTO2 gene exon 5 (A to G; rs156697) were determined using Taqman genotyping assay by RT-PCR. The results showed that the distribution of genotypes and alleles frequencies at rs156697 A>G polymorphism, The frequency of wild AA genotype was Highly Significant (P<0.01) lower in PCOS patients than in apparently healthy subjects (28% *versus* 70%, respectively, p<0.01). In contrast, the frequency of heterozygous AG genotype was highly significantly (p<.0.01) higher in PCOS patients when compared with apparently healthy subjects (64% *versus* 24%, respectively, χ^2 = =9.091, *OR*=1.62, p<0.01), The heterozygous AG genotype represent as a risk factor for PCOS incidence in Iraqi women, no significant differences in the frequency percentage of GG genotypes between apparently healthy subjects and PCOS patients. Hormonal analysis for LH, FSH, and Testosterone was performed by using Cobas e 411(automated principle). The results indicate that the mean serum LH significantly increase (P<0.05) in PCOS patients (7.26 ±1.37) mIU/mL compared with a mean of controls (4.32 ±0.39) mIU/mL. The results of FSH in the present study indicate that the mean of serum Testosterone was highly significantly (P<0.01) in PCOS patients (0.566 ±0.10) mIU/mL compared to the mean of controls (0.254 ±0.02) mIU/mL.

Keywords: polycystic ovary syndrome, GSTO2 Polymorphism, Glutathione S transferase omega 2.

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Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age, with a prevalence of 6–15 percent. Chronic anovulation and high levels of androgen may be associated with this disease, and these issues can lead to infertility. Patients with this syndrome may have a higher incidence of obesity, insulin resistance (50–70%), dyslipidemia, endothelial dysfunction, metabolic syndrome, and risk factors for type 2 diabetes mellitus (DM), however, the cause of this heterogeneous syndrome is unknown (1). Women with PCOS have at least two of the three requirements, according to the modified criteria stated at the Rotterdam meeting:(1) oligo ovulation or anovulation (2) higher levels of androgen (male hormones) in blood tests or through signs such as acne and extra hair growth on the face and body, and (3) an excessive number of follicles in ultrasound ovaries, with more than 12

follicles in each ovary measuring 29mm and/or increased ovarian volume (>10 mL) (2). Because polycystic ovary syndrome can manifest as a variety of diseases, the Rotterdam meeting criteria divided the disease into four phenotypes: (1) Frank or classic polycystic ovary syndrome, which includes chronic anovulation, hyperandrogenism, and polycystic ovaries, (2) Classic non-polycystic ovary syndrome, which includes chronic anovulation, hyperandrogenism, and normal ovaries, and(3) non-classic ovulatory PCOS, which is distinguished by regular menstrual cycles, hyperandrogenism, and polycystic ovaries. and (4) Non-classic or normoandrogenic PCOS, which involves chronic anovulation, normal androgen levels, and polycystic ovaries(3). Elslimani *et al*. (4) suggested that hormonal abnormalities related to subclinical hypothyroidism include elevated TSH levels and a higher LH-FSH ratio on ultrasonography, as well as polycystic ovaries. Zhu *et al*. (5) Patients with PCOS had greater BMI, E2, LH, and LH/FSH ratios when compared to controls. The current study was to look at a possible link between GSTO2 N142D (rs156697) and PCOS, Examine the connection between the prevalence of PCOS in Iraqi women, which is still under investigation. The GSTO2 polymorphism at nucleotide 424 (rs156697) converts asparagine to aspartate (N142D) (6). The aim of the current study was to investigate a possible relationship between *GSTO2* N142D (rs156697) polymorphisms and PCOS, which, to our knowledge, has not yet been studied.

Materials and methods

This study has been achieved at the Institute of Genetic Engineering and Biotechnology and Kamal Al-Samarrai infertility treatment Hospital in Baghdad during a period from November 2021 to January 2022. A total of 100 Iraqi women were separated into two groups for the study. There were 50 PCOS patients and 50 apparently healthy people in the study. All PCOS patients' medical histories were obtained, and the condition was first discovered with an ultrasound exam., If they had oligomenorrhea, amenorrhea, or severely irregular menses, information was noted., Patients range in age from 16 to 42 years old. Blood samples were collected from PCOS patients and healthy women by drawing 6 ml of venous blood from each., during (2 nd –4 th) day of the menstrual cycle; for individuals with a regular cycle, early follicular phase. Depending on the length of the cycle, blood samples were taken from patients with oligomenorrhea or anovulation. Each sample was divided into two tubes, with 2ml of whole blood placed in a tube containing EDTA (Ethylene Diamine Tetracetic Acid) for DNA extraction and genetic section and the remaining 4 ml of whole blood have been placed in a Serum-separating tube; serum was separated and isolated from this 4 ml of blood by centrifuging for 10 minutes at 3000 round per minutes (rpm), for hormonal tests (FSH, LH, Testosterone).

Biochemical test

Serum-Follicle Stimulating Hormone (FSH) and Serum-Luteinizing Hormone (LH) and Serum testosterone Hormone (T) By Cobas e 411.

Method

Five hundred µL of the sample was used in Cobas e411.for hormonal assay. (automated principle).

Genomic DNA isolation

Total genomic DNA was extracted

from entire fresh blood and stored in EDTA K3-containing tubes for molecular investigations. Geneaid DNA extraction kit (Bioneer, Korea). After obtaining genomic DNA, agarose gel electrophoresis was used to ensure that the extracted DNA was present and intact (7). and then Estimation of DNA Concentration and Purity A Nanodrop tool is used to determine the concentration of DNA samples. At 260 nm wavelength, 1 µl of extracted DNA was inserted into the lens of an apparatus to determine the concentration in ng/L.

The TaqMan probe contains a reporter dye (FAM and VIC) at the 5´end of the probe and a quencher dye (MGB) at the 3´ ends of the probe. The sequences of TaqMan fluorescent oligonucleotide probes and primers were prepared according to (8), synthesized by Alpha DNA Ltd (Canada), and stored lyophilized at (-23°C). Dilute 10µL of primers, probe, and stock solution in 90 µL of nuclease-free water and store at -23C until use to make a working solution with a concentration of 10M.

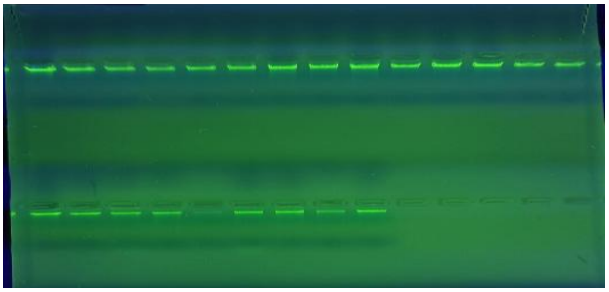

Figure (1): *GSTO1* AS-PCR products on a 2% agarose gel

Primers and probes

Table (1): Primer and probe used in the study

Primer/probe	Sequence (5′ →3′ direction)
GSTO2 gene,exon 5 ,chromosome 10 ,NG-023363.1, for rs156697	
Forward	**CCCACATTTGACCAAGGAGTG**
Reverse	**GCCTAGCTTCCTCCACTGTA**
FAM- probe	**TAATCTGAAGGCAGCCCTG**
VIC-probe	**CTGATCTGAAGGCAGCCCT**

DNA samples from PCOS patients were genotyped for the *GSTO1* gene SNPs (rs156697) with a Taqman SNP genotyping assay using a real-time thermocycler according to the protocol recommended by the manufacturer.

Table (2): Components of real-time PCR/ allelic discrimination

No.	Components	Volume	Final Conc.	Volume (µl)	
1	qPCR Master (PROBE)	10 µl	1X	10	
2	Forward primer	0.2-2.0 µl	0.1-1.0 µM	10 µl	3
3	Reverse primer	0.2-2.0 µl	0.1-1.0 µM	10 µl	
4	Fluorescence Probe	Variable	≤500ng/reaction	20 µl	
5	Template DNA	Variable	-	4	
6	Water, RNase free	Up0 to 20	-	3	

Table (3): Real time PCR program for *GSTO1* gene SNPs (rs4925)

Step	Temperature (ºC)	Duration	Cycles
Hold 1	50	2 min	1
Hold 2	95	10 min	1
Denature	95	10 sec	25-40
*Anneal	60	45 sec	25-40

In this step, the acquiring green and yellow (FAM and VIC) were added.

Statistical analysis

The Statistical Analysis System- (9) program was used to affect different factors in study parameters. Chi-square test was used for significant comparison between percentage and least significant difference –the LSD test was used for significant comparison between means in this study, and also odd ratio was used to determine risk factors (9).

Results

Genotypes and alleles frequency of rs156697 SNP at GSTO2 gene

The aim of the study GSTO2 gene polymorphism (rs156697 A>G) among Iraqi women with polycystic ovary syndrome and in apparently healthy controls. The genotypes and allele frequency distributions for rs156697 SNP polymorphism are presented in table (4). According to the findings, the frequency of the wild AA genotype was Highly Significant (P<0.01) lower in PCOS patients than in apparently healthy subjects (28% *versus* 70%, respectively, χ^2 = 9.00, *OR*=Ref, p<0.01). In contrast, the frequency of heterozygous AG genotype was highly significantly (p<.0.01) higher in PCOS patients when in comparison with apparently healthy subjects (64% *versus* 24%, respectively, χ^2 = 9.091, *OR*=1.62, p<0.01). We believe that the effect of the heterozygous AG genotype of *GSTO2* gene SNP (rs156697) in exon 5 on the susceptibility of the development of PCOS in Iraqi women could be better clarified with a large sample.

As shown in Table 4, no significant differences in the frequency percentage of GG genotypes between apparently healthy subjects and PCOS patients. The frequencies of A and G alleles were 0.60 and 0.40 in PCOS patients and 0.82 and 0.0.18 in apparently healthy subjects, respectively.

Table (4): Genotypes and alleles frequency of rs1156679 SNP GSTO2 gene in apparently healthy subjects *versus* Iraqi polycystic ovary syndrome patients.

Genotype (rs156697 [1])	PCOS PatientS [2] No (%)	Control [3] No (%)	Chi-Square (χ^2)	O.R. (C.I)
Wild: AA	14(28.00%)	35(70.00%)	9.00 **	Ref.
Heterozygous: AG	32(64.00%)	12(24.00%)	9.091 **	1.62 (0.92-3.57)
Mutant: GG	4(8.00%)	3(6.00%)	0.142 NS	0.319 (0.16-0.82)
Allele	Frequency			
A	0.60	0.82	--	--
G	0.40	0.18	--	--

NS: Non-Significant. **: <0.01 level of highly significance.
[1] SNP in GSTO1 gene; [2] PCOS = polycystic ovary syndrome healthy subjects;
[3] healthy subjects; X^2:chi square; OR: odds ratio; CI: confidence interval. N=50 for each group.

Hormonal assays

The results indicate that the mean serum LH significantly increase (P<0.05) in PCOS patients (7.26 ±1.37) mIU/mL compared with a mean of controls (4.32 ±0.39) mIU/mL, as shown in table (5).

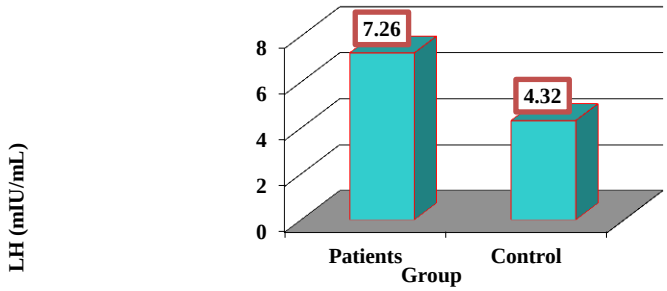

Figure (2): Comparison between PCOS patients and control groups in LH

The results of F S H i n the present study indicate that serum FSH concentrations were unaffected Overall.

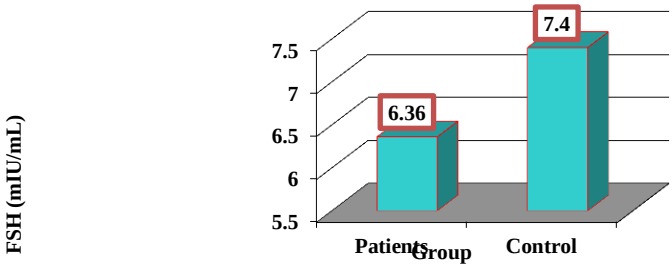

Figure (3): Comparison between PCOS patients and control groups in FSH

The results indicate that the mean of serum Testosterone was highly significantly (P<0.01) in PCOS patients (0.566 ±0.10) mIU/mL compared with a mean of controls (0.254 ±0.02) mIU/mL, as shown in table (5).

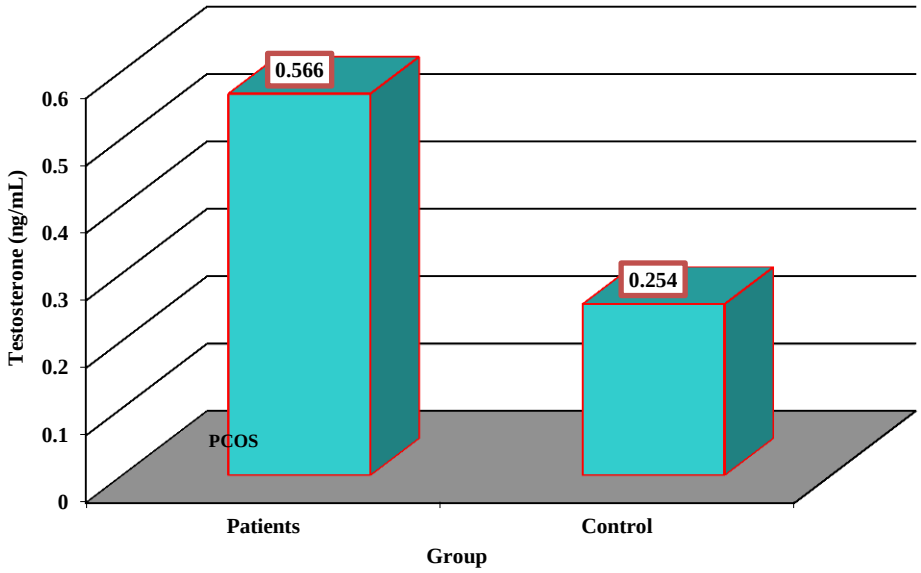

Figure (4): Comparison between PCOS patients and control groups in Testosterone

Table (5): Comparison between PCOS patients and control groups according to the selected hormonal profile

Group	Mean ± SE		
	LH (mIU/mL)	FSH (mIU/mL)	Testosterone (ng/mL)
PCOS [1]	7.26 ±1.37	6.36 ±0.53	0.566 ±0.10
Control [2]	4.32 ±0.39	7.40 ±0.56	0.254 ±0.02
T-test	2.862 *	1.550 NS	0.222 **
P-value	0.0441	0.184	0.0068

[1] Patient with polycystic ovary syndrome. and [2] healthy subjects. NS: No significant. *: Significant at 0.05 level. ** : <0.01 level of highly significance. (FSH): follicle-stimulating hormone, (LH): luteinizing hormone, (T): testosterone hormone.

Discussion

Genotypes and alleles frequency of rs156697 SNP at GSTO2 gene

This study examined polymorphisms of GSTO2gene (rs156697 A>G in exon 5) among Iraqi women who suffered from PCOS and apparently healthy as control and tested their association with the phenotype of PCOS at the Kamal Al-Samarrai infertility treatment Hospital in Baghdad.

To the best of our knowledge, this is the first study on the association of GSTO2 polymorphism with PCOS women. The study of Eshre and ASRM group, (10), their study involved long-term health risks related to polycystic ovary syndrome, and showed the GSTO disorder can cause increased production of oxygen free radicals induced by hyperandrogenism in the early stage of PCOS and may be associated with insulin resistance and other metabolic disorders associated with PCOS women. The Alpha GST-like enzyme is

found only in steroidal tissues and has 230-fold higher catalytic efficiency in 3-keto-steroids than 3β-hydroxysteroid dehydrogenase, so the decreased expression of this gene may lead to a decrease in the concentration of steroid hormones (11). The study of GSTO enzymes, which can form disulfide bonds with GSH, plays important role in cell resistance to oxidative damage. They are also involved in regulating the biosynthesis and transport of hormones within cells. A study has been conducted on omega-GST class polymorphisms in malignant diseases. GSTO2 gene polymorphisms are associated with bladder, breast, and ovarian cancer (12). However, not reported no relationship between polymorphisms of GSTO2 and PCOS. In addition to its role in infertility disorders, PCOS has been identified as the underlying cause of a variety of reproductive cancers, including endometrial malignancies, and ovarian and breast cancers. A study of (13), has shown that carriers of the GSTO2 D142 genotype are at risk of developing hypothyroidism. These findings could be related to the role of the GSTO2 enzyme in the detoxification of metabolites. The current results investigated the wild type and heterozygotes were high recurrent in control than in PCOS, while allele A was a high frequency in healthy subjects than in PCOS patients.

Hormonal assays

LH hormones were significantly higher in-patient groups (Rotterdam ESHRE/ ASRM, 2004). High levels of LH not only affect oocyte maturity and human reproduction but also lower fertility and higher miscarriage prevalence (14). The present results of serum LH concentrations agree with the previous studies, (15, 16, 17) since they found that the mean values of serum LH of patients are significantly higher than those of controls.

The results of the present study agree with the results of previous studies, (18, 19, 20) since they have observed that the mean values of patients' serum FSH are significantly lower than those of controls. (21) have found lower FSH concentrations in postmenopausal women with PCOS than apparently healthy controls. (22) have found that Saudi women with PCOS had lower FSH concentrations versus controls. Recently,(23) found that Iraqi women with PCOS and PCOS plus T2DM had lower FSH levels than apparently healthy subjects.

The results of serum testosterone in the present study agree with that of (24, 25, 26) since they concluded that the mean values of the patient's serum T were significantly higher than those of controls.

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