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Effect of CYP2D6*10 (100C > T) Polymorphisms on Clomiphene Citrate Response in Iraqi Women with PCOS

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Abstract

Background

Clomiphene citrate (CC) is a commonly prescribed drug to induce ovulation in women with PCOS, a hormonal disorder affecting women of reproductive age. The CYP2D6 is an enzyme vital in metabolizing CC to its active metabolite. Variations in this gene, such as CYP2D6*10, which reduces enzyme activity, can affect drug metabolism and potentially impact treatment outcomes.

Materials and Methods

The cohort study, conducted from September 2023 to April 2024, enrolled 80 women diagnosed with PCOS. All patients received 100mg/day of CC from cycle day 2 for at least 2 cycles. Whole blood was collected for hormonal assays and CYP2D6*10 genotyping by ARMS PCR. Furthermore, an ultrasound was performed to determine follicle size and endometrial thickness during the cycle.

Results

We found that the frequency of CYP2D6*10 genotypes is 66.3% (**CC**), 27.5% (**CT**), and 6.3% (**TT**). Women with the mutant allele exhibited significantly lower concentrations of the active metabolite and higher concentrations of the prodrug ($p < 0.05$). At the same time, the CT genotype showed a higher AMH level.

Conclusions

Our findings prove an association between the CYP2D6*10 genotype and CC metabolism. However, further research with a larger sample size to confirm these findings. Additionally, an assessment of AMH level may also help predict CC.

تأثير التغايرات الجينية لـ(100C>T) CYP2D6*10 على استجابة عقار الكلومفين ستريت في النساء اللواتي يعانون من متلازمة تكيس المبايض

مريم حسين الهر, اثير مجيد رشيد, حسن محمود ابو المعالي

الخلاصة

المقدمة

كلوميفين سيتريت هو دواء يستخدم عادة كالخط الأول لعلاج تحفيز الإباضة لدى النساء المصابات بمتلازمة تكيس المبايض، وهو اضطراب هرموني يؤثر على النساء في سن الإنجاب. يعد إنزيم CYP2D6 حيويًا في استقلاب سيترات الكلوميفين إلى مستقلبه النشط. يمكن أن تؤثر الاختلافات في هذا الجين، مثل **CYP2D6*10**، المعروفة بتقليل نشاط الإنزيم، على استقلاب الدواء وربما تؤثر على نتائج العلاج.

المواد وطريقة العمل

أجريت الدراسة الأترابية في الفترة من سبتمبر 2023 إلى أبريل 2024 وسجلت 80 امرأة مصابة بمتلازمة تكيس المبايض. تلقى جميع المرضى 100 ملغ يوميا من كلوميفين ستريت من اليوم الثاني للدورة و لمدة دورتين على الأقل. تم جمع الدم لعمل الفحوصات الهرمونية والتنميط الجيني لـ(**CYP2D6*10**) بواسطة ARMS PCR. علاوة على ذلك، تم إجراء الموجات فوق الصوتية لتحديد حجم جريب المبيض وسمك بطانة الرحم خلال فتره العلاج.

النتائج

لقد وجدنا أن تواتر اختلافات **CYP2D6*10** هو 66.3% (النوع السائد **CC**)، و27.5% (متغاير الزيجوت **CT**)، و6.3% (المتغاير الطافر **TT**). أظهرت النساء ذوات الأليل الطافر تركيزات أقل بكثير من المستقلب النشط وتركيزات أعلى من الدواء الأولي ($P < 0.05$). في حين لم يتم العثور على فروق ذات دلالة إحصائية في الهرمونات الإنجابية، ولكن كانت مستويات AMH أعلى في النمط الجيني CT.

الاستنتاج

تثبت النتائج التي توصلنا إليها وجود علاقة بين النمط الوراثي **CYP2D6 * 10** واستقلاب عقار كلوميفين ستريت. ومع ذلك، فإن إجراء المزيد من الأبحاث باستخدام حجم عينة أكبر يجب أن يؤكد هذه النتائج ويوضح الآثار السريرية. بالإضافة إلى ذلك، قد يساعد تقييم مستوى AMH أيضًا في التنبؤ باستجابة عقار كلوميفين.

1. Introduction

Polycystic ovarian syndrome (PCOS) is one of the most prevalent reproductive endocrine disorders in women. This condition is complicated by inadequate treatment regimens, delayed diagnosis, and diagnostic difficulties(Hoeger et al., 2020). Infertility is brought on by this syndrome(Patel, 2018). One of the oldest medications that is still the treatment of choice for inducing ovulation in patients with PCOS is clomiphene citrate (CC)(Bashir et al., 2021). CC is a selective estrogen receptor modulator (SERM) that exists as (Z)-clomiphene and (E)-clomiphene(Euler et al., 2022). Inducing estrogenic and anti-estrogenic effects, the medication binds specifically to estrogen receptors in the ovary, endometrium, cervix, and hypothalamus resulting in the inhibition of negative estrogenic feedback, thus increasing gonadotropins which increase the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH)(Feh and Wadhwa, 2022). CYP2D6 is an enzyme that metabolizes E-clomiphene and converts it into even more potent metabolites called E-4-hydroxy-clomiphene (E-4-OH-CLO). About 8–54% of women are not responsive to clomiphene treatment, and characteristics like obesity and hyperandrogenemia influence response variability. Furthermore, studies have demonstrated the significance of the highly polymorphic CYP2D6 enzyme in (E)-Clomiphene bio-activation (Kovar et al., 2022). The frequency of the CYP2D6 allele differs widely between ethnic and ancestral groups. The CYP2D6*10 (rs1065852) 100C > T polymorphism leads to the substitution of proline to serine and causes an mRNA splicing defect, producing an IM phenotype(El Akil et al., 2022). This study aims to investigate CYP2D6*10 genotypes and their association with CC response and metabolism.

2. Patients and Methods

2.1. Patients

80 PCOS women who had been unable to naturally conceive and were diagnosed with PCOS according to the updated Rotterdam consensus (Al-Asadi, 2024) were recruited from the infertility outpatient clinic from the period of September 1, 2023, to April 1, 2024, at Gynecological and Obstetric Hospital in Kerbala province, Iraq. PCOS women take clomiphene citrate 100 mg/ dose for more than one cycle and obtain written consent from participants after explaining the study's purpose and requiring them to complete a designed questionnaire. Patients were excluded if they had started clomiphene citrate therapy concurrently with an insulin sensitizer agent, lipid-lowering agent, or any other ovulation induction therapy. Other reasons for exclusion include endocrine disorder, History of untreated thyroid, adrenal disorders, or pituitary dysfunction. Other causes of infertility like Male or tubal factors were also excluded.

2.2. Materials

Clomiphene citrate (Clomid) was acquired from a commercial pharmacy. FSH, LH, estradiol, prolactin (PRL), and anti-Müllerian hormone (AMH) kits were obtained from Roche in Germany. Materials for genetic analysis, including the gSYNC™ DNA Extraction Kit, DNA ladder marker, Master Mix, and primers were purchased from Geneaid (Taiwan), Bioneer (Korea), BioLabs (USA), and Macrogen (Korea), respectively.

2.3. Methods

2.3.1. Study Design

A cohort study was conducted from September 2023 to April 2024. Before starting medication, participants were enrolled on the second day of their menstrual cycle. Follow-up assessments were performed on the twelfth day of at least two consecutive menstrual cycles after drug administration. Data collected included age, BMI, menarche, family history of other diseases, Clomiphene dosage, hirsutism, and regularity of menstruation. A specialist doctor performed ultrasound examinations to measure follicle size and endometrial thickness and Hormone levels were also assessed on cycle day 12.

2.3.2. Blood Collection

Blood samples were collected from each participant on cycle day 2 (before clomiphene) and cycle day 12 (after clomiphene). Approximately 6 mL of blood was drawn into K3 EDTA tubes for genomic DNA extraction and Gel & Clot Activator Blood Collection Tubes for hormonal analysis. Blood samples were centrifuged at 5000 rpm for 10 minutes to obtain plasma for hormonal level measurement and to assess drug and metabolite concentrations.

2.3.3. Assessment of Reproductive Hormones

The levels of FSH, LH, estradiol (E2), and AMH in the blood were measured on the 2nd and 12th days of the menstrual cycle using a Cobas e 411 Analyzer from Roche, Germany.

2.3.4. Determination of Concentrations Of E-Clomiphene and Its Metabolite

E-Clomiphene (E-CLO) and its metabolite E-4-OH-CLO were measured at the zist Gene Baft Lab in Tehran, Iran, using a SCIEX 4500 QTrap LC/MS/MS apparatus. Standard samples were prepared by dissolving them in a solution of 5% acetic acid and acetonitrile. A standard solution containing 10 ppm was introduced into the LC/MS/MS apparatus, and an 80:20 ratio of acetonitrile to water was used as the mobile phase. To identify unknown metabolites, the device was scanned from mass 360 to 450. The interface gas temperature was set to 500°C. A standard curve was constructed using serial dilutions of standard samples and ImageJ software to quantify metabolite concentrations based on the peak area ratio(Ganchev et al., 2011).

2.3.5. Genotyping Analysis

DNA was isolated from 200 µL of peripheral whole blood Using gSYNC™ DNA Extraction Kit (Geneaid/Taiwan) according to standard protocol. Extracted DNA was stored at −20°C before analysis. Genetic variation was examined of the metabolizing enzymes CYP2D6, we performed amplification refractory mutation system-polymerase chain reactions (ARMS-PCR). We included primers to amplify CYP2D6*10 100C>T (rs1065852) which was designed in (Hinrichs et al., 2007). Table1 provides information about the intended set of primers, including product sizes.

Table1: Sequence of the Primers for the Variant CYP2D6*10 with Product Size

SNPs	Primer sequence (5 ’->3 ’)	Product size
2D6*10 F out	GGG GCA AGA ACC TCT GGA GC	505 bp
2D6*10 R out	CTG GTC CAG CCT GTG GTT TC	
2D6*10 R WT	AGT GGC AGG GGG CCT GGA GG	351 bp
2D6*10 F*10	ACG CTG GGC TGC ACG CTT CT	192 bp
F out= outer forward primer, R out= outer reverse primer, R WT= reverse wildtype primer, F*10= Mutant primer		

Following several iterations of optimization, the PCR mixture contained 8 µL of OneTaq Quick-load 2X Master Mix (New England BioLabs/ USA), 2µL of extracted DNA, 1 µl from 2D6*10 F out primer, and 1 µl from 2D6*10 R out primer, and for 2D6*10 R WT and 2D6*10 F*10 primers, 1 µl of each were added to separate PCR tube as the volume completed to 20 µl with 7 µL of ddH2Othe. Final optimized thermal cycle conditions for this variation were as follows: 5 min at 95 °C of initial denaturation, followed by 35 PCR cycles of 30 sec at 95 °C (denaturation), 30 sec at 63 °C (annealing temperature), and 1 min at 72 °C (extension) then the final extension flowed 10 min at 72 °C. PCR products were separated based on 2% agarose gel at 45 V for 1 h and visualized by ethidium bromide under a UV illuminator. The 505bp represents the internal control while 351bp is an indication of a wildtype allele and 192bp is an indication of a mutant allele.

2.4. Statistical Analysis

Statistical analyses were conducted using SPSS 26. To determine whether the data are normally distributed, we conduct normality tests (Shapiro-Wilk test). The Wilcoxon Signed-Rank Test was utilized to compare study variables before and after treatment during menstruation. The Kruskal-Wallis test was employed to analyze differences among CYP2D6*10 genotypes (CC, CT, and TT). This non-parametric test is suitable for analyzing groups when the normality is unmet. Chi-square tests were used to assess categorical results. A P-value < 0.05 was considered statistical significance. It is worth mentioning that small sample sizes can introduce increased variability, reduce statistical power, and hinder the detection of true differences between groups.

3. Results

The study examined hormonal changes with endometrial and follicle development during treatment in the menstrual cycle. Table2 displays that estradiol (E2), LH, and the LH/FSH ratio significantly increased (P<0.05), while FSH remained relatively stable (P>0.05). Additionally, there were significant increases in follicle size and endometrial thickness at P=0.001.

Table2: Study Variables Before and After Treatment (Data Present as Median +IQR)

Parameter	Median +IQR (before treatment)	Median +IQR (after treatment)	*P*-value	Conclusion
E2	35.6+29.3	75.65+80.63	0.001	Significant
FSH	5.93+4.075	5.75+2.657	0.127	Not significant
LH	7. 85+5.76	14.95+7.485	< 0.001	Significant
LH/FSH ratio	1.3+1.27	2.3+2.0	0.001	Significant
Follicle size	6.0+2.0	16.0+8.75	0.001	Significant
ET	4.5+1.50	8.0+3.0	0.001	Significant
E2: Estradiol, FSH: follicle stimulating hormone, LH: luteinizing hormone, ET: endometrial thickness				

3.1. Distribution of CYP2D6*10

ARMS PCR was utilized to identify the CYP2D6*10 variation. The gel imaging results showed gel band patterns in Fig.1.

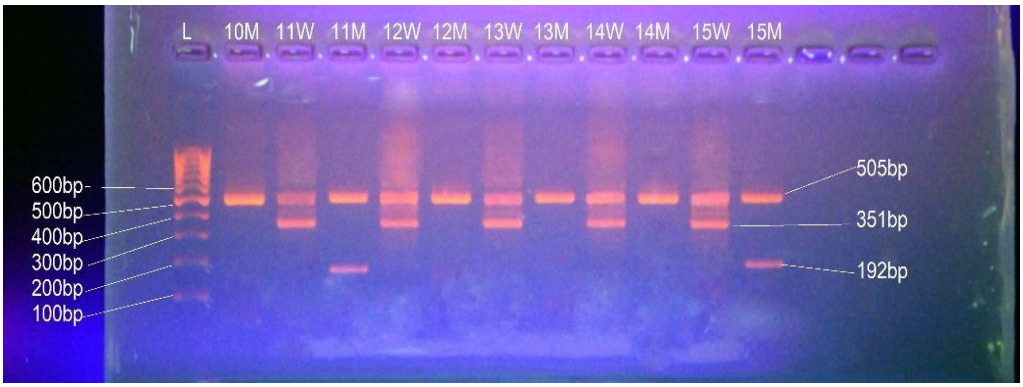

Figure 1: Image of CYP2D6*10 (100 C>T) Genotyping Using ARMS-PCR
L refers to DNA Ladder; M Indicates the Mutant Allele and W Indicates the Wildtype Allele.

It was found that the Frequency of the CYP2D6*10 allele in PCOS women carrying homozygous genotype **(CC)** is about 53(66.3%) and heterozygous genotype **(CT)** is 22(27.5%), while homozygous for the minor allele **(TT)** are 5(6.3%) as shown in the Fig.2

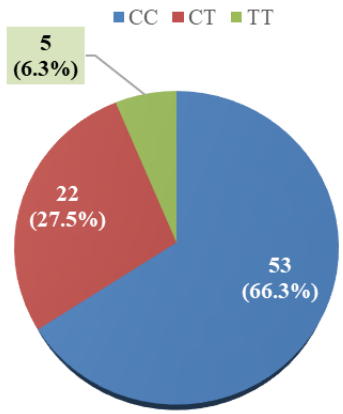

Figure2: Allele Genotypes Frequency of CYP2D6*10 for All Studied Women (N=80)
CC Represents Wildtype, CT Represents Heterozygous Carrier and TT Represents Homozygous Mutant.

3.2. Demographic Data

Demographic data including age, BMI, menarche, and marital duration were assessed in PCOS women. TT genotype showed older age (30y), earlier menarche (11) and higher BMI (72.2) but no significant differences were observed

compared to other genotype regarding these demographic factors (P > 0.05). Hirsutism and menstrual irregularity were assessed using the Chi-square test. Results for both hirsutism and menstrual irregularity were relatively similar (P > 0.05). as presented in Table3.

Table3: Demographic Data of PCOS Women Categorize According to Genetic Polymorphism (Data Present as Median + IQR and No (%))

Variables		Alleles of CYP2D6*10 100C > T			*P* – value
		CC (n=53)	CT (n=22)	TT (n=5)	
Age (y)		27+10.5	25+6.5	30+12.5	0.771
BMI (Kg/m^2)		26.2+6.1	27.3+2.1	27.2+7.6	0.907
Menarche (y)		12+1.5	12.5+1	11+4	0.278
Duration of Marriage (y)		6+4	4.5+1	3+10	0.928
Hirsutism	**No (n=40)**	26(49.1%)	11 (50%)	3 (60%)	0.896
	Yes (n=40)	27 (50.9%)	11 (50%)	2 (40%)	
Menstrual Regularity	**No (n=31)**	19 (35.8%)	9 (70.83%)	3 (60%)	0.554
	Yes (n=49)	34 (64.2%)	13 (29.1%)	2 (40%)	

3.3. Effects of CYP2D6*10 Genotypes on Hormonal Levels

The research examined the impact of CYP2D6*10 genotypes on reproductive hormones. Although there were variations in LH, LH/FSH ratio, and estrogen levels, none of these variances were statistically significant (P > 0.05). However, AMH levels exhibited a noteworthy increase in the CT genotype (3.5) compared to CC and TT at (P=0.002), as indicated in Table4.

Table4: CYP2D6*10 Genotypes Effect of Reproductive Hormones (Data Present as Median + IQR)

Variables	Alleles of CYP2D6*10 100C > T			Kruskal-Wallis H	P-vale
	CC (n=53)	CT (n=22)	TT (n=5)		
AMH (ng/ml)	2.5+1.8	3.5+2.1	3.1+1.5	12.108	0.002
FSH (mIU /ml)	5.9+3.4	5.4+4.7	5+2.3	0.77	0.68
LH (mIU /ml)	15+8.6	15.1+8.1	11.1+3.4	3.614	0.164
LH/FSH ratio	2.2+2.3	3.1+2	1.8+1	3.353	0.187
E2 (pg/ml)	75+88.3	64.2+68.4	125.4+127.2	3.562	0.168
AMH: Anti-Müllerian hormone, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol					

3.4. Effect of CYP2D6*10 Genetic Polymorphism on Ultrasound

The impact of CYP2D6*10 genotypes on follicle size and endometrial thickness was investigated, and the findings are shown in Table 5. The median follicle sizes and endometrial thickness were similar across all genotypes, and no statistically significant differences were observed (P > 0.05).

Table5: Influence of CYP2D6*10 Polymorphisms on Ultrasound (Data Present as Median + IQR)

Variables	Alleles of CYP2D6*10 100C > T			Kruskal-Wallis H	P-value
	CC (n=53)	CT (n=22)	TT (n=5)		
Follicle Size (mm)	16+9	16.5+9.6	18+7	0.481	0.786
ET (mm)	8+3	8+1.5	8.5+3.3	0.705	0.703
ET= Endometrial Thickness					

3.5. Effects of CYP2D6*10 Polymorphism on E-Clomiphene Citrate And E-4-Hydroxyclomiphene Levels

As concentrations of drug (E-CLO) and its active metabolite E-4-hydroxy-clomiphene (4-OH-CLO) were measured at cycle day 12 to analyze the effect across different CYP2D6*10 genotypes, it had been observed that E-CLO levels were significantly higher in the TT genotype compared to CT and CC genotypes at $P<0.05$ and E-4-OH-CLO levels also differed significantly with CT and CC genotypes having higher levels than the TT genotype ($P<0.05$). These findings suggest that CYP2D6*10 genotypes influence the metabolism of E-clomiphene. Table6.

Table6: Effect of CYP2D6*10 Polymorphisms on Drug and Its Metabolite (Data Present as Median + IQR)

Variables	Alleles of CYP2D6*10 100C > T			Kruskal-Wallis H	*P* – value
	CC (n=53)	CT (n=22)	TT (n=5)		
E-CLO (nM)	3.4+0.5	3.4+0.5	3.9+0.4	10.786	0.005
E-4-OH-CLO (nM)	4.4+0.7	4.2+0.5	2.8+0.5	14.451	0.001

4. Discussion

Clomiphene citrate (CC) is a drug used for ovulation induction, especially for PCOS. It contains a mixture of unequal isomers known as E-clomiphene and Z-clomiphene (Rostami-Hodjegan et al., 2004).CC is a non-steroidal that demonstrates estrogenic agonist and estrogenic antagonist properties (Gadalla et al., 2018). CC demonstrates varying efficacy in inducing ovulation, with success rates ranging from 73% at 50 to 150 mg doses. Despite these overall positive outcomes, individual responses to CC can differ. While factors predicting resistance to CC are currently unclear, the inconsistent findings across studies hinder the accurate prediction of ovulation induction failure(Kane, 2021). CYP2D6 is important in metabolizing clomiphene as it acts primarily on E-clomiphene and converts it into active metabolites (Ghobadi et al., 2008). Previous research has indicated that the CYP2D6*10 (100C>T, rs1065852) polymorphism is related to an IM status which carries a higher risk of side effects or non-reaction to pro-drugs. The **(C)** allele represents the wild-type, while the **(T)** allele is a variant(Orengo-Mercado et al., 2013). The CYP2D6*10 allele differs among populations and ethnic groups, some Asian populations have a higher frequency of the gene than do those with European ancestry(Gaedigk et al., 2017). Since our study provides useful data on the CYP2D6*10 genotype frequency in Iraqi PCOS women, direct comparisons with other populations are limited due to the lack of published data from many countries. Our finding reveals a high frequency of the wild-type **CC** genotype, with a lower frequency of the **CT** and even lower mutant **TT** genotypes. This suggests that many PCOS women have normal CYP2D6 enzyme activity. Previous studies of Korean PCOS women have identified allele*10 as the most frequent allele(Ji et al., 2016) A similar study involving random volunteers in Korea also reported comparable findings(Kim et al., 2018). On the other hand, The CYP2D6*10 allele frequency varies across different populations in the Middle East and North Africa. Iran had the highest frequency of this allele at 20.4%, followed by Jordan and Turkey with frequencies of 14.8% and 13.14%, respectively. The lowest frequencies were observed in the United Arab Emirates, Saudi Arabia, and Syria, ranging from 3.3%, 3%, and 2.94% respectively(Khalaj et al., 2019). While it was reported in Iraq at a frequency of 13.4%. Arabs exhibited a higher frequency of normal metabolizers (NMs) compared to Europeans, East Asians, and Americans, with rates of 70.53% in Arabs, 51.05% in Europeans, 51.91% in East Asians, and 63.6% in Americans(Alali et al., 2022).

The study focused on women with PCOS and prolonged infertility, aiming to understand the potential role of genetic factors in this condition. All participants had a similar age of menarche, suggesting that the CYP2D6 polymorphisms studied do not influence menarche onset. Obesity is commonly associated with PCOS (Messinis et al., 2015), the study found no significant differences in BMI among the genotypes, indicating that obesity in PCOS is not linked to CYP2D6*10 variations and it has been proposed that women with a higher BMI may require larger doses of Clomiphene due to their potential resistance to the medication (Ghobadi et al., 2009). Hirsutism is a frequent occurrence in women with PCOS(Oliveira and Comim, 2024) half of the participated PCOS women had it. Irregular periods are also one of the most common features of PCOS resulting from elevation in free testosterone caused by obesity(Mari et al., 2023) Both show no relation with CYP2D6*10 polymorphism. In conclusion, this study did not find a significant association between the CYP2D6*10 genotype and demographic characteristics in PCOS women.

This study demonstrates the effectiveness of CC therapy in inducing ovulation and preparing the reproductive system for pregnancy in women with PCOS. Our study suggests that the CYP2D6*10 genotype may influence AMH levels in women with PCOS. Women with the **CT** genotype, carrying one variant allele, exhibited significantly higher AMH levels than those with the **CC** (wild-type) or **TT** (mutant) genotypes. AMH is a biomarker of ovarian reserve and can affect follicular development and response to ovulation induction therapies(Peluso et al., 2014). Higher AMH levels have been associated with reduced sensitivity to FSH and increased resistance to CC treatment(Garg and Tal, 2016). Therefore, the observed association between CYP2D6*10 genotype and AMH levels may partially explain the variability in response to CC therapy among PCOS patients. The significant increases in LH, LH/FSH ratio, follicle size, and endometrial thickness observed after treatment are consistent with the known mechanisms of action of CC. The increase in E2 levels is likely a secondary effect of the increased LH, as LH stimulates ovarian follicle development and estrogen production(Holesh et al., 2017). The significant increase in LH levels is a key factor in inducing ovulation. The increase in follicle size is essential for the development of a mature egg that can be released during ovulation and thickened endometrium is necessary for the implantation of a fertilized egg (Rachmawati et al., 2023). Overall, these findings highlight the effectiveness of CC therapy in PCOS. Although no significant differences were found in these reproductive hormones (FSH, LH, LH/FSH ratio, and E2) between the three genotypes. Furthermore, the results of this study indicate that the CYP2D6*10 genotype may not notably affect follicle size or endometrial thickness in women with PCOS undergoing CC therapy. Although there were minor differences in these parameters among the genotypes, they did not show statistical significance. Several factors, including sample size, other genetic polymorphisms, or factors influencing follicle development and endometrial thickness in PCOS, could contribute to the absence of a clear association between the CYP2D6*10 genotype and ultrasound parameters.

Lastly, Previous studies have demonstrated that the CYP2D6*10 allele is associated with a reduced-function phenotype, leading to decreased enzyme activity (Kane, 2021), and according to the proposed theory, individuals with an intermediate metabolizer (IM) phenotype were expected to have lower levels of active drug metabolites. Another study reported a direct correlation between the amount of CYP2D6 present and the extent of E-clomiphene metabolism (Ghobadi et al., 2008). Our study implies that the CYP2D6*10 genotype can influence the metabolism of CC in women with PCOS. Patients with the **TT** genotype, carrying two variant alleles, have high levels of the parent drug (E-CLO) and lower levels of the active metabolite (E-4-OH-CLO). These findings align with a Korean study that

identified significant differences in parent drug concentration between the wild-type and mutant genotypes. Additionally, the elimination half-life and total drug exposure of E-4-OH-CLO were significantly longer in individuals with the mutant genotype compared to those with other genetic variations (Kim et al., 2018). Similarly, another study identified a correlation between CYP2D6*10 genotypes and plasma concentrations. Plasma levels of the active metabolites were 8 times higher than the (PM) women, who had parent drug concentrations (E-CLO) 6 times higher(Mürdter et al., 2012).

This study has some limitations, such as the relatively small sample size, particularly for the TT genotype, which may have limited the statistical power to detect significant differences in reproductive hormones.

5. Conclusion

In conclusion, the metabolite concentrations were significantly changed across the genotypes although the reproductive hormones and ultrasound were not, and thus these findings highlight the complexity of genetic influences on these different parameters and suggest that other genetic, environmental, or lifestyle factors may be more relevant in understanding variations in these traits. Further research could explore additional genetic markers or larger populations to confirm these observations.

6. Ethical Approval

The research was conducted in Kerbala city and was reviewed and approved by the Training and Human Development Center/research committee in Kerbala with approval number 2023180. All patients provided informed consent before they participated in the study.

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