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ORIGINAL ARTICLE

The Role of CYP450 in Women with Polycystic Ovary Syndrome and Elevated Gonadal Hormone and DHEA Concentrations

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ABSTRACT

The polycystic ovary syndrome (PCOS) is a complex endocrine disorder characterized by polycystic ovarian morphology, hyperandrogenism and ovulatory dysfunctions. One of the key molecular contributors to its pathophysiology is the altered activity of enzymes belonging to the Cytochrome P450 (CYP450) superfamily, which plays a central role in steroid hormone biosynthesis and metabolism. This study was designed to be a case-control study conducted in the Kamal Al Sammarae hospital for fertility and IVF during the period from 1 August 2024 to 1 September 2025. In this study, 100 blood samples were collected from PCOS women aged between (20-35) years who were enrolled in this study. These women were complaining of polycystic ovary syndrome (PCOS), documented according to the Rotterdam criteria. Also, 100 Non-PCOS women were selected as a control group; the non-PCOS control group matched with the PCOS group by age and BMI. The results of gonadal hormone analysis in PCOS cases revealed that the mean level of TSH was (2.62± 0.12) compared to the control group (1.81±0.08), and the mean level of LH in the patient group was (7.78±1.12) in comparison to the controls (3.72±0.54). On the other hand, FSH levels were (5.94±0.21) in the PCOS women compared to the healthy control (1.97±0.12) and the mean level of Free T3 was (0.61±0.05) compared to the controls (0.01±0.03) with highly significant variations (P≤0.01) respectively. In addition, the mean level of Prolactin in PCOS women was (12.22± 0.71) in comparison to the controls (11.73±0.35) with a non-significant difference (P=0.53). The mean levels of Insulin in POCS women was (12.85±1.10) compared to the controls (13.43±0.36) with a non-significant variation (P=0.61), while the mean level of FBS in the patient group was (7.95±0.37) compared to the healthy group (0.23±0.14), and the mean level of HOMAIR was (5.43± 0.86) in comparison to the controls (1.58±0.22), with highly significant differences (P≤0.01) respectively. Mutations were detected in rs6466, rs6474, rs147821751, and rs6467 genes, and GG was changed to GA of G/A heterozygous allele in samples 1,5,6,7,8,12,15,16,18 and 19. While it was changed to AA of A/C heterozygous allele in samples 3 and 17 of rs6466 in comparison to the controls. However, the CC were the CC nucleotides were changed to CT sequence of C/T heterozygous allele.

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1- INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex endocrine disease characterized by polycystic ovarian morphology, hyperandrogenism, and ovulatory dysfunction. Altered activity of enzymes in the cytochrome P450 (CYP450) superfamily is one of the molecular factors involved in its pathophysiology, since these enzymes have central roles in steroid hormone biosynthesis and metabolism. CYP21A2, commonly referred to as CYP21, is of particular interest because it encodes 21-hydroxylase, the main enzyme involved in aldosterone and cortisol synthesis in the adrenal cortex [1]. Changes in the activity of CYP450 enzymes, including CYP17A1, CYP11A1, CYP19A1, and CYP21A2, can disrupt normal steroidogenesis and contribute to the hormonal imbalance characteristic of PCOS. Hyperandrogenism is a defining feature of PCOS and is generally associated with increased androgen levels, including testosterone and dehydroepiandrosterone (DHEA). CYP17A1 is primarily associated with increased androgen production, whereas CYP21A2 has a modulatory role [2]. Partial or deficient CYP21A2 activity, including in non-classical or mild forms, can reduce cortisol synthesis and trigger compensatory increases in adrenocorticotropic hormone (ACTH) production [3]. ACTH then stimulates adrenal androgen production, contributing to higher DHEA levels and symptoms that overlap with PCOS [4]. The interaction of pituitary hormones, including LH and prolactin, thyroid function reflected by TSH, and antioxidant systems such as glutathione (GSH), helps shape the biochemical and clinical features of PCOS. In women with PCOS, luteinizing hormone (LH) levels are often elevated, which commonly increases the LH/FSH ratio [5,6]. This imbalance stimulates the ovarian theca cells to produce more androgens, contributing to anovulation, hyperandrogenism, and menstrual irregularity. Thus, elevated LH is a major driver of ovarian dysfunction in PCOS. Thyroid-stimulating hormone (TSH) is another important factor because thyroid dysfunction, particularly subclinical hypothyroidism, is frequently observed in women with PCOS [7]. Higher TSH levels may worsen metabolic problems, including weight gain and insulin resistance, while also intensifying menstrual irregularities. Since thyroid hormones affect ovarian function both directly and indirectly, their imbalance is a significant concern in PCOS management [8].

Most women with PCOS experience insulin resistance at some point, even without diabetes. This may lead to irregular periods, weight gain particularly around the abdomen acne, hair thinning, or excessive facial hair [9]. IR can also develop into acquired or genetic conditions such as obesity, noninsulin-dependent diabetes, polycystic ovary syndrome, and metabolic syndrome (10). Insulin controls blood glucose levels by being secreted into the bloodstream (11). The relationship between vitamin D3 (VD3) and PCOS matters, yet it is often overlooked. Rather than functioning solely as a vitamin, vitamin D helps regulate hormones, and a deficiency may affect both the metabolic and reproductive features of PCOS [12]. This study examines the pathophysiological role of Cytochrome P450 (CYP450) enzyme activity in women with Polycystic Ovary Syndrome (PCOS), focusing on its association with increased gonadal hormones and Dehydroepiandrosterone (DHEA) concentrations. It also compares metabolic, hormonal, and oxidative stress markers including insulin, TSH, LH, FSH, GSH, and Vitamin D3 in PCOS patients and healthy controls. A further aim is to identify potential genetic variations (mutations) in the CYP21A2 gene that may help explain the molecular basis of the disorder.

2- MATERIALS AND METHODS

This study was designed to be a case-control study conducted in the Kamal Al Sammarae hospital for fertility and IVF during the period from 1 August 2024 to 1 September 2025. İn the current study, (100) blood samples were collected from PCOS women aged between (20-35) years who were enrolled in this study. These women were complaining of polycystic ovary syndrome (PCOS), documented according to the Rotterdam criteria. Also, (100) Non-PCOS women were selected as a control group; the non-PCOS control group matched with the PCOS group by age and BMI. Fasting blood sugar, Insulin, TSH, LH, Free T3, Prolactine and VD3 were tested by Automated 411 E Cobas Analyzer. While CYP450, CYP21, DHEA and GSH were examined by the Enzym Immuno-Assy ELISA technique. The molecullar analysis was performed with PCR technique and sequenced sanger sequener.

Kits	Company/ Origin
ReliaPrep™ Blood gDNA Miniprep System, Agarose, Ethidium Bromide Solution (10mg/ml), GoTag Green Master Mix, Nuclease Free Water, TAE 40X, Quantifluor dsDNA System, 100bp DNA ladder	Promega, USA
Primers	Macrogen, Korea

Primers

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)	Product size (bp)
CYP21A2-F	CCCAGGCTGGTCTTAAATTC	60	929
CYP21A2-R	CGATGTGATCCCTCTTCTCTA		

Ethical approval

Before the study commenced, written informed consent was obtained from all participants. Ethical approval was granted by the Medical Ethics Committee (Approval No. 204) on April 1, 2026.

Statistical analysis

The notation "mean ± SE" is commonly used to present a sample statistic, typically the mean, together with its standard error (SE). This format shows the sample average and its associated precision, providing an estimate of how closely the sample mean reflects the true population mean and serving as a basis for constructing confidence intervals.

3- RESULTS AND DISCUSSION

Table 1 showed the distrbution of poly cystic ovary was 37 (37%) in the age groups (20-24) years and (25-29) years which was equal to the control group, with non-signifiifican variations (P= 1.0). But the distrbution in the age group (>29) years was 26(26%) with a non-significant variation (P=1.0).

Table (1):Distribution of studied groups according to age groups (years)

Age groups/years	PCOS (n=100)		Control (n=100)		*P-value
	N	%	N	%	
(20-24)	37	37	37	37	**1.0
(25-29)	37	37	37		
>29	26	26	26	26	
Total	100	100	100	100	

***Chi-square test was applied to calculated P-value, **non-significant**

Data showed that the distribution of polycystic ovary syndrome (PCOS) across the different age groups was identical to that of the control group, with no statistically significant differences (P = 1.0). In women aged 20–24 years, 37 cases (37%) were reported, which was exactly the same proportion as in the control group (37%). Similarly, in the 25–29 year age group, the distribution remained equal between PCOS cases and controls. For women older than 29 years, the proportion was 26 (26%) in both the PCOS and control groups. A P-value of 1.0 indicates that there is absolutely no difference between the groups in terms of age distribution. Statistically, this means that age (within these categories) was not associated with the occurrence of PCOS in your study population. The identical percentages suggest that the groups were well matched by age, which is methodologically important because it reduces age as a potential confounding factor. Although PCOS is commonly diagnosed in women of reproductive age (especially in their 20s), the findings indicate that, in this sample, the condition was evenly distributed across the studied age ranges when compared with controls. Therefore, age did not appear to influence the prevalence of PCOS in this dataset. In conclusion, the absence of significant variation (P = 1.0) confirms that there is no statistical association between age group and PCOS in your study, and the control group was appropriately age-matched [13]. The prevalence of PCOS according to the Cities showed that in Baghdad was 86(86%), Salahaldin and Diyala 4(4%), Babylon, Al-Diwanya and Anbar was 1(1%), while in Wasit was 2(2%). One the other hand, the distribution of PCOS according to residency showed that it was 69(69%) in main cities and in the side cities was 31(31%). Moreover, the distribution of PCOS according to the occupation showed that it was 92(92%) among housewives and 8(8%) among employed women with no significant differences (P= 1.0, 1.0 and 1.0) respectively as shown in table 2.

Table (2): Distribution of the studied groups according to city type and residency status

		PCOS (n=100)		Control (n=100)		*P-value
		N	%	N	%	
City name	Baghdad	86	86	86	86	1.0
	Salahaldin	4	4	4	4	
	Diqar	1	1	1	1	
	Diyala	4	4	4	4	
	Babylon	1	1	1	1	
	Wasit	2	2	2	2	
	Al-Diwanya	1	1	1	1	
	Anbar	1	1	1	1	
	Total	100	100	100	100	
Residency	City	69	69	69	69	**1.0
	Cityside	31	31	31	31	
	Total	100	100	100	100	
Occupation	Housewife	92	92	91	91	**1.0
	Employed	8	8	9	9	
	Total	100	100	100	100	

***Fisher exact probability was used, **Chi-square test was applied to calculate P-value**

The findings demonstrated that the majority of PCOS cases were from Baghdad, accounting for 86% of the total sample, while much lower percentages were observed in Salahaldin and Diyala (4%), Wasit (2%), and Babylon, Al-Diwanya, and Anbar (1% each). This difference may be largely attributable to population density and accesses to the healthcare facilities, i.e. Baghdad is the capital city and is highly densely populated with a more access to specialized endocrinology and gynecological facilities. Therefore, people who reside in Baghdad are likely to have higher diagnosis rates in comparison with women in other cities, where healthcare accesses are more limited. Kamber *et al.,* (2025) showed that the greater incidence in Baghdad reflects better detections rather than actual epidemiological differences [14]. Results of residency showed that 69% of PCOS patients were residing city (urban) areas, whereas 31% were residing sub-city (rural) areas, and this difference possibly related to lifestyle issues usually seen in urban residents, e.g. dietary habit, sedentary behaviors, psychological stresses and increased obesity rates, all of which are known to be PCOS risk factors. In addition, women residing in urban areas likely look for medical consultations, participating in the elevated recorded proportions [15].

With regard to occupation, most of the cases were housewives (92%), whereas 8% were employed women. However, it was revealed that there was no significant difference (P = 1.0) between PCOS prevalence and occupation, suggesting no significant association between PCOS occurrence and occupation. The higher housewife percentage may be reflecting the general cultural structures and demographics of the cases rather than causal relationship indication [16]. Numerical variations were reported across cities, occupation and residency by Yu *et al.,* (2025). With no statistically significant variations (P = 1.0) indicating that such parameters were not significantly related to PCOS in this study. Larger, multicenter studies are recommended to further investigate geographical and sociodemographic influences on PCOS prevalence while controlling for confounding factors such as BMI, lifestyle, and access to healthcare [17]. The prevalence of PCOS according to family history showed that 31(31%) have family history and 69(69%) have none. Furthermore, the smoker patients was 5(5%) with no significant variations P=1.0 1.0 respectively. On the other hand, there was 21 (21%) with Hypotension compared to 79(79%) with normal blood pressure, with significant variations P ≤0.01. In addition, the body mass index BMI (Kg/m2) among women with POCS cases between (18.5-24.9) was 11(11%), between (25-29.9) was 32(32%). Also, between (30-34.9) was 19(19%), While, in >40 was 6(6%) with no significant differences P=0.93, as shown in table 3.

Table (3): Distribution of studied groups according to family history, smoking habits and BMI (Kg/m2) scores

		PCOS (n=100)		Control (n=100)		
		N	%	N	%	*P-value
Family history	Yes	31	31	31	31	*1.0
	No	69	69	69	69	
	Total	100	100	100	100	
Smoking	Yes	5	5	5	5	*1.0
	No	95	95	95	95	
	Total	100	100	100	100	
Blood pressure	Normal	79	79	100	100	*≤0.01
	Hypotension	21	21	0	0	
	Total	100	100	100	100	
BMI (Kg/m2)	18.5-24.9	11	11	9	9	*0.93
	25-29.9	32	32	32	32	
	30-34.9	31	31	31	31	
	35-39.9	19	19	18	18	
	>40	6	6	9	9	
	Total	100	100	100	100	

***Chi-square test was applied to calculate P-value**

The findings of this study showed that 31% of women with PCOS reported a positive family history, while 69% had no family history. Although a positive family history was present in nearly one-third of the participants, the majority did not report affected relatives. This suggests that while genetic predisposition may contribute to PCOS development, environmental and lifestyle factors may also play an important role in its occurrence. Our results were in agreement with (Begum *et al.,* 2024) who found similar results [18]. Tao *et al.,* (2021) demonstrated that only 5% of cases were smokers in comparison with 95% non-smokers, with non-significant associations (P=1.0), suggesting that smoking is not significantly associated with prevalence of PCOS in the study. The small number of smokers may limit the capacity for detection of any potential relationship [19].

Results of blood pressure showed significant difference ($P \leq 0.01$), as 21% of cases were hypotensive, while 79% showed normal blood pressure, and such significant P-value indicates possible blood pressure association with PCOS in those participants. Nevertheless, because most of cases showed normal blood pressure, additional investigations are necessary for determining the clinical significance of hypotension in women with PCOS. These findings were in agreement with (Silva *et al.,* 2024) who indicated that (PCOS) is the endocrine disease that most commonly affects women at reproductive age, with significant effects for cardio metabolic health [20]. Milanese *et al.,* (2025) revealed in terms of BMI that the majority of cases were obese or overweight. Specifically, 11% had normal BMI (18.5–24.9 kg/m²), 32% were overweight (25–29.9 kg/m²), 19% were in obesity class I (30–34.9 kg/m²), and 6% had morbid obesity (>40 kg/m²). A non-significant difference was found (P = 0.93), in spite of the higher rates of obese and overweight patients, and such lack of significance may be attributed to distribution pattern or sample's size. However, this high rate of obese and overweight patients aligned with the existing literature suggesting that excessive body weights are often related to PCOS and may cause exacerbation of its reproductive and metabolic features [21].

It was shown in the study that the BMI and family history were commonly observed in PCOS women, but blood pressure demonstrated a statistically significant correlation. Such results highlight the multifactorial natures of PCOS and the significance of observing metabolic, genetic and cardiovascular factors to evaluate and manage it (Shruthi *et al.,* 2025) [22]. It was revealed in Table 4 that the mean level of TSH was (2.62± 0.12) compared to the control group (1.81±0.08), and the mean level of LH in the patient group was (7.78±1.12) in comparison to the controls (3.72±0.54). On the other hand, FSH levels were (5.94±0.21) in the PCOS women compared to the healthy control (1.97±0.12) and the mean level of Free T3 was (0.61±0.05) compared to the controls (0.01±0.03) with highly significant variations (P≤0.01) respectively. In addition, the mean level of Prolactin in PCOS women was (12.22± 0.71) in comparison to the controls (11.73±0.35) with a non-significant difference (P=0.53).

Table (4): The mean levels of gonadal hormones between cases and control

Test	Group	Mean	SE	t-test	P-value
TSH(mIU/L)	PCOs	2.62	0.12	5.35	≤0.01
	Control	1.81	0.08		
LH(IU/L)	PCOs	7.78	1.12	3.24	≤0.01
	Control	3.72	0.54		
FSH(IU/L)	PCOs	5.94	0.21	16.2	≤0.01
	Control	1.97	0.12		
Free T3(pg/mL)	PCOs	0.61	0.05	7.981	≤0.01
	Control	0.01	0.03		
Prolactin(ng/mL)	PCOs	12.22	0.71	0.62	0.53
	Control	11.73	0.35		

The results showed clear endocrine changes in PCOS women in comparison with the controls, with most of the hormones showing statistically significant variations ($P \leq 0.01$), except the prolactin hormone. Though both values are within the normal reference value, the increase of TSH in patients may indicate a tendency towards altered thyroid functions or subclinical hypothyroidism. Such results agreed with (Khan *et al.,* 2025) who showed that thyroid dysfunctions may exacerbate menstrual irregularity, anovulation as well as metabolic disturbance, commonly seen in PCOS [23]. Akhter *et al.,* (2025) demonstrated that such finding is consistent with the classic endocrine hallmarks in PCOS i.e. elevated secretion of LH because of altered regulation of hypothalamic–pituitary–ovarian axis. High levels of LH result in stimulation of the ovarian theca cells for producing more androgens, participating in anovulation, hyperandrogenism as well as follicular arrests [24]. The level of FSH was also significantly increased among PCOS women (5.94±0.21) in comparison with the control group (1.97±0.12). Despite this elevation of FSH, Xia *et al.,* (2023) indicated that in several cases of PCOS the LH/FSH ratio elevated because of a relatively greater increased LH level. The normal follicular ovulation and maturation is impaired by disruption of the balance between FSH and LH, resulting in infertility [25]. Elevated level of Free T3 can reflect an associated thyroid abnormality or a compensatory metabolic mechanism. Thyroid hormones affect insulin sensitivity, ovarian functions and metabolic rates, which are usually disturbed in patients with PCOS.

The findings indicate that prolactin does not appear to play a major role in the endocrine profile of the studied PCOS group. While mild hyperprolactinemia can sometimes coexist with PCOS, it is not a defining hormonal feature in most cases (Kizmaz *et al.,* 2025) [26]. The findings support that PCOS is associated with significant disturbances in Pituitary gonadotropins (elevated LH and altered LH/FSH balance) and Thyroid function parameters (elevated TSH and Free T3). These hormonal imbalances contribute to anovulation, menstrual irregularities, hyperandrogenism, infertility and possible metabolic dysfunction. (Palomba *et al.,* 2023), concluded that the lack of significant change in prolactin suggests that it is not a primary contributor in this cohort [27]. Kukreti *et al.,* (2025) demonstrated that the results reinforce the concept that PCOS is a complex endocrine disorder involving both reproductive and thyroid-related hormonal alterations [28]. The mean levels of Insulin in POCS women was (12.85±1.10) compared to the controls (13.43±0.36) with non-significant variations P=0.61, while the level of FBS was (7.95±0.37) compared to the healthy group (0.23±0.14), and the mean level of HOMAIR was (5.43± 0.86) in comparison to the controls (1.58±0.22) with a highly significant differences $P \leq 0.01$ respectively, as shown in table 6.

Table (5): The mean level of diabetic screening tests between cases and controls

Test	Group	Mean	SE	t-test	P-value
Insulin (µIU/mL)	PCOs	12.85	1.10	0.50	0.61
	Control	13.43	0.36		
FBS (mg/dl)	PCOs	7.95	0.37	14.23	≤0.01
	Control	0.23	0.14		
HOMAIR	PCOs	5.43	0.86	4.27	≤0.01
	Control	1.59	0.22		

It was observed by Kaczmarczyk *et al.,* (2025) that it might be surprising as PCOS is usually related to hyperinsulinemia. Nevertheless, the insulin level alone may be confusing. The secretion of insulin is dynamic and affected by fasting status, timing and compensatory mechanism. Thus, the normal level of insulin does not exclude underlying insulin resistances [29]. Moreover, Ahmed *et al.,* (2025) recommended looking at the fasting blood sugar level now. The PCOS women showed a much greater mean (7.95±0.37) n comparison with the control group (2.23±0.14), with a highly significant variation (P ≤0.01), which indicates impaired glucose regulations in PCOS women. Although the absolute control value is observed to be unusually decreased (depending on unit), dysglycemia indicated in PCOS, which is the key takeaway and the strong statistical difference [30]. However, Li *et al.,* (2023) reported that because HOMA-IR reflected insulin resistance by combining insulin with fasting glucose, which highly supports the insulin resistance in patients with PCOS. Clinically, this aligns with the well-established role of insulin resistance in PCOS pathophysiology. Insulin resistance contributes to hyperandrogenism, ovarian dysfunction, and increased risk of type2 diabetes and metabolic syndrome [31].

Table 6 showed that the mean level of CYP450 with PCOS patients was (248.57±10.53) compared to the control group (68.71±8.04) with higher significant variations P≤0.01. In addition, the mean level of CPY21 was (2.32±0.13) compared to the control group (2.32±0.15) with no significant differences. Moreover, the mean level of DHEA was (150.62±5.20) compared to the control group (42.87±8.04) with higher significant variations P≤0.01. Furthermore, the level of GSH was (26.66±0.71) compared to the control group (13.38±0.42) with higher significant variations P≤0.01. Finally, the level of Vit-D3 was (15.38±1.19) compared to the control group (48.45±1.63) with higher significant variations P≤0.01.

Table (6): The mean levels of some hormones and enzymes between cases and controls

Test	Group	Mean	SE	t-test	P-value
CYP450(pg/ml)	PCOs	248.57	10.53	13.57	≤0.01
	Control	68.71	8.04		
CPY21 (pg/ml)	PCOs	2.32	0.13	0.03	0.97
	Control	2.32	0.15		
DHEA (µg/dL)	PCOs	150.62	5.20	17.49	≤0.01
	Control	42.87	3.28		
GSH(IU/mL)	PCOs	26.66	0.71	15.93	≤0.01
	Control	13.38	0.42		
Vit-D3(ng/ml)	PCOs	15.38	1.19	16.33	≤0.01
	Control	48.45	1.63		

The results indicate clear biochemical differences between women with polycystic ovary syndrome (PCOS) and the control group, reflecting alterations in steroidogenesis, oxidative stress status, and vitamin metabolism. The elevated CYP450 level in PCOS patients may reflect increased activity of steroidogenic pathways, which is commonly associated with hyperandrogenism in PCOS. The enhanced activity of CYP450 may result in elevated production of androgen, participating in clinical manifestations like acne, hirsutism and menstrual irregularity, as shown by (Rambaran *et al.,* 2025), [32]. On the other hand, the no significant difference in CYP21 is shown to be involved in the synthesis of cortisol and aldosterone in the adrenal cortex. In addition, Abdolmonem *et al.,* (2025) concluded that the absence of significant variations indicates that this enzyme may not have an important role in the PCOS pathophysiology in these studied women, which also enhances the difference between PCOS and congenital adrenal hyperplasia (CAH), where the deficiency of CYP21 is a fundamental factor [33].

DHEA is the precursor of adrenal androgen which is involved in excessive androgen. The increased levels of DHEA in PCOS is coincided with former studies that showed elevated secretion of adrenal androgen, and this elevation may further cause worsening of hyperandrogenic symptoms and disruption of normal ovarian follicular developments [34]. The high levels of GSH may be the compensatory responses to oxidative stresses, which are commonly found in PCOS women. Increased oxidative stress was associated to insulin resistances, inflammations and impaired ovarian functions in patients with PCOS [31]. Deficiency of Vitamin D is often related to PCOS and may be involved in metabolic disturbance, such as obesity, insulin resistance and hormonal imbalances, vitamin D reduces insulin sensitivity by increasing glucose concentration and insulin resistance (35). Decreased levels of Vitamin D can also negatively influence reproductive functions and follicular developments. These results indicate that PCOS is related to enhanced activity of steroidogenic enzymes, high productions of androgen, altered balance of oxidative stress in addition to deficiency of Vitamin D [33]. As the increase of CYP450 and DHEA demonstrate

the role of abnormal steroid metabolisms, the unaltered level of CYP21 indicates that all steroidogenic enzymes are not involved. Moreover, Wang *et al.*, (2025) revealed that the high levels of GSH suggests adaptation of oxidative stress, while low Vitamin D3 can be involved in reproductive and metabolic dysfunction in PCOS. These biochemical alterations could provide insight into the underlying mechanisms of PCOS and may help guide future therapeutic strategies [34].

Amplification of CYP21A2 gene of human samples were fractionated on 1.5 agarose gel electrophoresis and stained by Ethidium bromide as shown in figure 1.

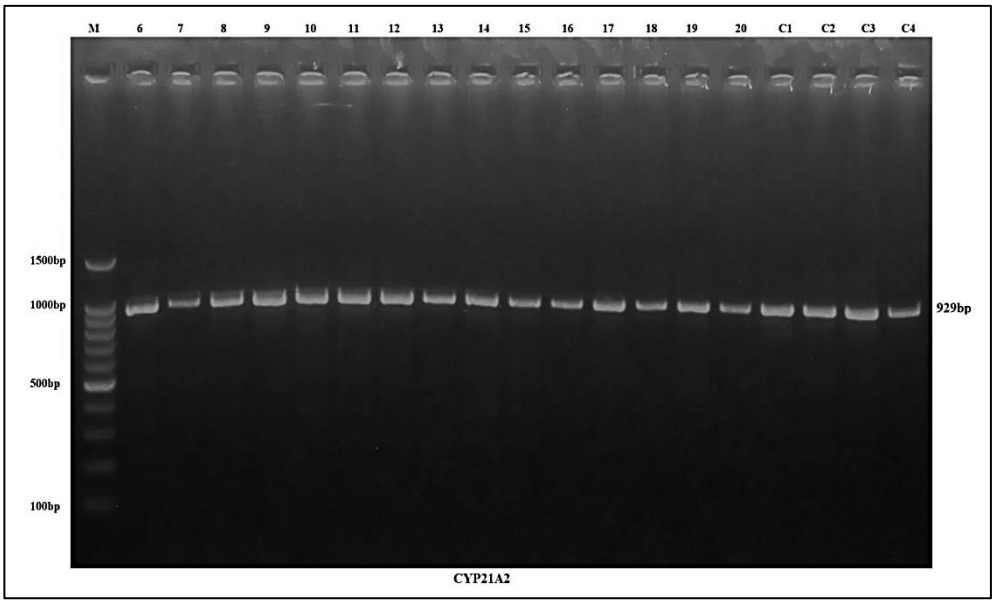

Fig (1): Amplification products of the CYP21A2 gene region separated on 1.5% agarose gel electrophoresis and stained with ethidium bromide. M: 100 bp DNA ladder; Lane's 6-C4 show 929 bp PCR products

Figures 2,3,4, and 5 revealed that a mutations occurrence in rs6466, rs6474, rs147821751 and rs6467 genes, GG was changed to GA in samples 1,5,6,7,8,12,15,16,18 and 19, while it was changed to AA in samples 3 and 17 of rs6466 compared, to the control group. On the other hand, the nucleotides CC were changed to CT in samples 1, 6, 7,8,10,11,12,18 19 and 20 with no change to TT of rs6474gene. Moreover, CC was changed to CT in the sample only with no change in others in rs147821751 gene. But the change occurred in rs6467 gene, the GG transported to GT in samples 5,7,10 and 14 while the GG transported to the TT in samples 3, 8, 9,11,12,13,15,16,17 and 18 in comparison to the controls.

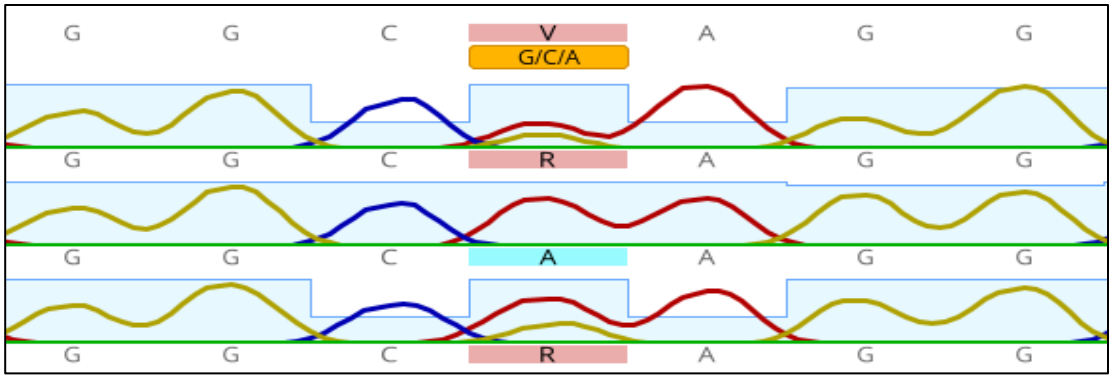

Fig (2): Analysis of SNP *rs6466* of CYP21A2 gene using Sanger sequencing Presence of the "G" and "A" peak indicative of G/A heterozygous allele

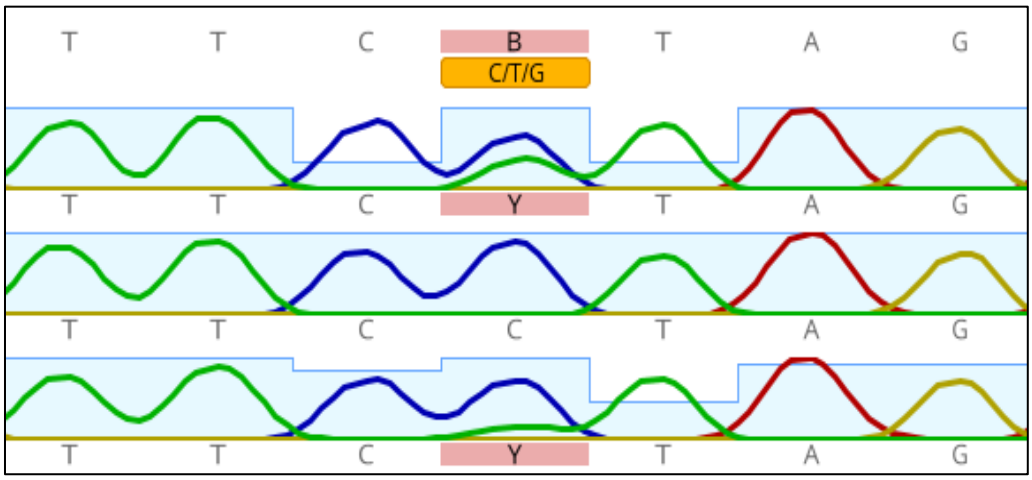

Fig (3): Analysis of SNP *rs6474* of CYP21A2 gene using Sanger sequencing. Presence of the "C" and "T" peak indicative of C/T heterozygous allele

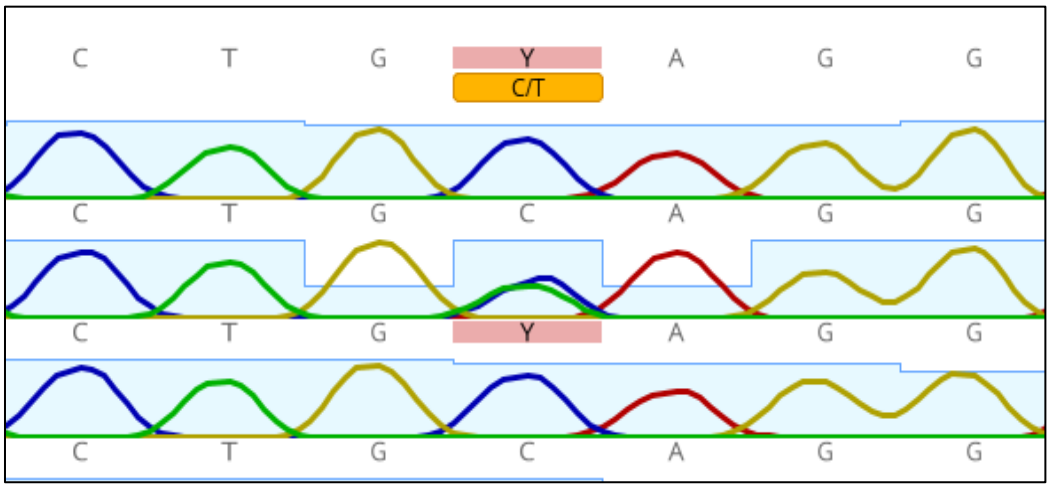

Fig (4): Analysis of SNP *rs147821751* of CYP21A2 gene using Sanger sequencing Presence of the "C" and "T" peak indicative of C/T heterozygous allele

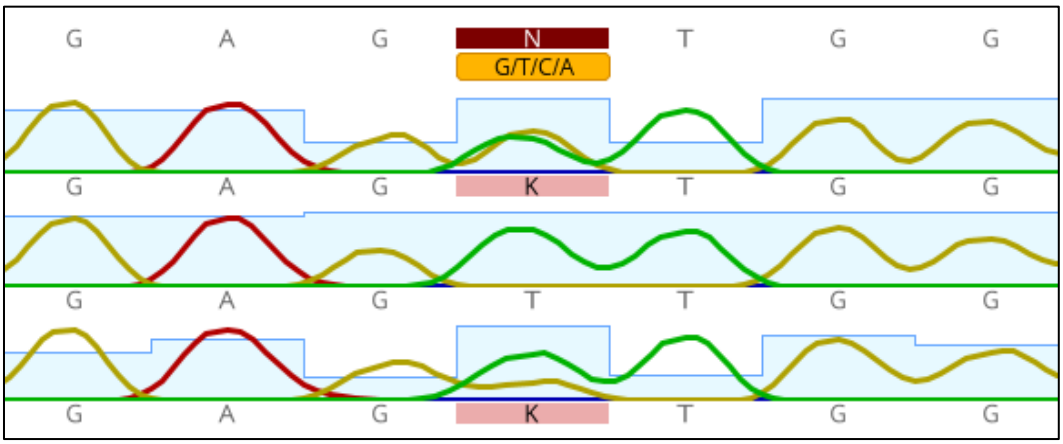

Fig (5): Analysis of SNP *rs6467* of CYP21A2 gene using Sanger sequencing Presence of the "G" and "T" peak indicative of G/T heterozygous allele

The present analysis revealed several nucleotide variations across four SNP loci (rs6466, rs6474, rs147821751, and rs6467) when compared with the control genotype. For rs6466, the control genotype (GG) showed polymorphic changes in multiple samples. A heterozygous mutation (GG→GA) indicating a relatively frequent occurrence of the A allele within the studied population. In addition, a homozygous mutant genotype (GG→AA) was detected in these samples. This is what was shown by Joseph *et al., (*2025) who reported that the presence of both heterozygous and homozygous variants suggests that rs6466 may exhibit notable allelic variability and could potentially influence gene function depending on its genomic location (coding, regulatory, or intronic region). These results were in a harmony with. Jahantigh, D and Hosseinzadeh A evaluated the association between genetic polymorphisms of XRCC5 VNTR, XRCC6 -61C>G, and XRCC7 6721G>T with male infertility susceptibility [36]. In the rs6474 locus, the reference genotype (CC) was altered to CT in samples 1, 6, 7, 8, 10, 11, 12, 18, 19, and 20. However, no TT homozygous mutant genotype was observed in the examined samples. This pattern indicates that while the mutant T allele is present in the population, it occurs predominantly in the heterozygous form. The absence of the TT genotype might suggest a lower allele frequency or possible selective constraints against the homozygous mutant form. Chai *et al.,* concluded that patients with *CYP3A5* rs15524 and *CYP3A7* rs776744 mutants had a higher IPV. Furthermore, the mortality caused by rejection in wild-type patients and mutant patients of CYP3A7 rs776744 was 4.5% vs. 7.7% ($p = 0.564$), (Jahantigh *et al.,* 2025) [37].

Regarding rs147821751, only a single heterozygous variation (CC→CT) was detected in one sample, while all remaining samples retained the wild-type CC genotype. This result indicates that such SNP is relatively rare in the study sample, suggesting decreased genetic variabilities in this locus (Chai *et al.,* 2025) [38]. On the other hand, rs6467 showed the highest variation degree in the studied SNPs, and this was achieved by Alharazy *et al.,* (2025) who evidenced that the wild-type of genotype GG was changed to GT in the samples 5, 7, 10 & 14, suggesting the presence of heterozygosity. Furthermore, a homozygous mutant genotype (GG→TT) was observed in samples 3, 8, 9, 11, 12, 13, 15, 16, 17, and 18. The high TT genotype frequency indicates that the mutant alleles might be relatively commonly found in the study sample and might have clinical or functional implication based on the biological roles of such loci [36]. Such findings demonstrated different mutation frequencies within the four SNPs, with rs6467 demonstrating the uppermost polymorphic distributions, then the rs6466 and rs6474, whereas rs147821751 showed minimal variations. These polymorphisms can be involved in genetic diversities and may potentially influence gene expressions, protein functions or vulnerability to diseases, necessitating more investigations with larger specimen size and functional analysis [39].

4- CONCLUSION

The results concluded that the alterations in the Cytochrome P450 (CYP450) systems, especially enzymes contributed to steroidogenesis, are involved in significant increase of androgen productions in women with Polycystic Ovary Syndrome, as observed by increased levels of DHEA. Increased levels of insulin suggest insulin resistance, which causes enhancement of the synthesis of ovarian androgen via CYP450 enzyme activity's stimulation, thus deteriorating hyperandrogenism and metabolic disturbance. The combined increased CYP450 activities, DHEA, insulin and thyroid hormone, as well as changed VD3 and body mass, reveals that PCOS is a multifactorial disease including complicated interaction between metabolic, genetic and endocrine pathways. In women with PCOS, thyroid dysfunctions are indicated by increased TSH, T3, T4 and Free T3, which might interact with reproductive and metabolic hormones, causing more ovarian function disruption.

REFERENCES

[1] Abdelmonem, B. H., Abdelaal, N. M., Rashwan, E. K. E. A., Hussein, A. A., Ahmed, M. A., Khashana, Y. F., Hanna, R. K., Hanna, M. M., and Abdelnaser, A. (2024). Decoding the role of CYP450 enzymes in metabolism and disease: A comprehensive review. *Biomedicines, 12*(7), 1467. https://doi.org/10.3390/biomedicines12071467

[2] Aboelros, S. A., Azab, H., Abo Elroose, A., and Hassan, N. R. (2025). Association of CYP21 gene polymorphism rs13405728, CYP11A1 gene polymorphism rs4077582 and insulin receptor gene rs2059806 in polycystic ovarian syndrome patients in Egypt: A pilot study. *Suez Canal University Medical Journal*, *28*(5), 9–26.

[3] Claahsen, H. L., van Grinten, H., Speiser, P. W., Ahmed, S. F., Arlt, W., Auchus, R. J., Falhammar, H., et al. (2022). Congenital adrenal hyperplasia—Current insights in pathophysiology, diagnostics, and management. *Endocrine Reviews, 43*(1), 91–159. https://doi.org/10.1210/endrev/bnab016

[4] Elhassan, Y. S., Hawley, J. M., Cussen, L., Abbara, A., Clarke, S. A., Kempegowda, P., et al. (2025). Society for Endocrinology clinical practice guideline for the evaluation of androgen excess in women. *Clinical Endocrinology, 103*(4), 540–566. https://doi.org/10.1111/cen.15265

[5] Zhang, Y., and Zhao, Z. C. H. (2025). Polycystic ovary syndrome: A metabolic disorder with therapeutic opportunities. *Cell Metabolism, 37*(10), 1932–1949. https://doi.org/10.1016/j.cmet.2025.08.002

[6] Al-Jawadi, Z. A. M., and Al-Taie, F. K. (2025). Clinical study of the role of thyroid stimulating hormone on polycystic ovary syndrome. *Health Biotechnology and Biopharma, 9*(2), 85–98. https://doi.org/10.22034/HBB.2025.20

[7] Khan, W. G., Azha, R., Ahmed, S., Bashir, S., and Ullah, F. (n.d.). Thyroid disorders in women with polycystic ovary syndrome. *Journal of Health, Wellness, and Community Research*. https://doi.org/10.61919/7w2kpm23

[8] Krysiak, R., Kowalcze, K., Burgio, J. O. S., Zaami, S., and Okopień, B. (2025). Polycystic ovary syndrome attenuates TSH-lowering effect of metformin in young women with subclinical hypothyroidism. *Pharmaceuticals, 18*(8), 1149. https://doi.org/10.3390/ph18081149

[9] Purwar, A., and Nagpure, S. (2022). Insulin resistance in polycystic ovarian syndrome. *Cureus, 14*(10), e30351. https://doi.org/10.7759/cureus.30351

[10] Hassan, A. J., Mohammed, N. S., Shweash, M., and Hadeed, H. M. (2020). Impact of anti-*Toxoplasma gondii* and adipose hormones with insulin resistant on obese aborted women. *International Journal of Drug Delivery Technology, 10*(1), 114–118. https://doi.org/10.25258/ijddt.v10i1.25

[11] Hasan, A. M., Hassan, A. S., and Atiyah, A. H. (2023). Study of diabetic imbalance during acute phase and recovery in patients with COVID-19 in Baghdad/Iraq. *Journal of Medicinal Chemistry Sciences, 6*(3), 515–521. https://doi.org/10.26655/JMCHEMSCI.2023.3.8

[12] Abow, F. H., Alsarraf, Z. H., and Ali, H. J. (2024). The role of vitamin D deficiency in pathogenesis of PCOS: A case-control study among females in Mosul City. *Iraqi Journal of Medical Sciences, 22*(2), 239–251. https://doi.org/10.22578/IJMS.22.2.8

[13] Kheirollahi, A., Hamidi, H., and Vatannejad, A. (2025). Distribution of polycystic ovary syndrome (PCOS) phenotypes in Iranian women: A cross-sectional study. *BMC Research Notes, 18*, 215. https://doi.org/10.1186/s13104-025-07202-6

[14] Kamber, H. M., Hussein, W. A., Al-Sattam, Z. M. J., Al-Jawdah, K., Muter, S., and Al-Musawi, S. (2025). Polycystic ovary syndrome and urinary tract stones: A body mass index-adjusted analysis in Iraqi women. *Al-Rafidain Journal of Medical Sciences, 8*(1). https://doi.org/10.54133/ajms.v8i1.1725

[15] Talukder, A., Tithi, T. A., Rumi, M. H., and Muyeed, A. (2025). Polycystic ovary syndrome and psychological distress: Urban–rural comparison among reproductive-aged women in Bangladesh. *PLOS Global Public Health*. https://doi.org/10.1371/journal.pgph.0004937

[16] Alenzi, A. O., Alqntash, N. H., Almajed, E. H., and AlZabin, A. K. (2024). Risk of polycystic ovary syndrome: A population-based analysis of sociodemographic factors, healthcare access, health behaviors, and health status. *BMC Women's Health, 24*, 623. https://doi.org/10.1186/s12905-024-03446-9

[17] Guo, Y., Ling, Y., Su, Y., and Li, Z. (2025). Analysis of factors influencing polycystic ovary syndrome in women of reproductive age based on directed acyclic graphs. *Scientific Reports, 15*, 37074. https://doi.org/10.1038/s41598-025-20980-0

[18] Begum, G. S., Almashaikhi, N. A. T., Albalushi, M. Y., Hajar, H. M., Alazawi, R. S., Goud, B. K. M., and Dube, R. (2024). Prevalence of polycystic ovary syndrome (PCOS) and its associated risk factors among

medical students in two countries. *International Journal of Environmental Research and Public Health, 21*(9), 1165. https://doi.org/10.3390/ijerph21091165

[19] Tao, Y., Liu, B., Chen, Y., Hu, Y., Zhu, R., Ye, D., Mao, Y., and Sun, X. (2021). Genetically predicted cigarette smoking in relation to risk of polycystic ovary syndrome. *Clinical Epidemiology, 13*, 527–532. https://doi.org/10.2147/CLEP.S311785

[20] Briceño Silva, G. D., Thomas Garcia, K. D., Ajamyan, H., Shekhawat, P., Rodriguez, L. C., Hammoud, A., Avalos Zapata, M. J., Flores Hernandez, N., and Rayon Rayon, H. M. (2024). Polycystic ovarian syndrome: Exploring hypertension and cardiometabolic implications. *Cureus, 16*(10), e70958. https://doi.org/10.7759/cureus.70958

[21] Milanese, C., Itani, L., Cavedon, V., and El Ghoch, M. (2025). The WHO BMI system misclassifies weight status in adults from the general population in North Italy: A DXA-based assessment study (18–98 years). *Nutrients, 17*(13), 2162. https://doi.org/10.3390/nu17132162

[22] Lakshmi, S. S., Meena, G., T. S., and Washington, J. K. (2025). Prevalence and predictors of metabolic syndrome in women with polycystic ovarian syndrome: A cross-sectional study. *Cureus,* 17(12), e98869. https://doi.org/10.7759/cureus.98869

[23] Khan, W. G., Azhar, R., Ahmed, S., Bashir, S., and Ullah, F. (2025). Thyroid disorders in women with polycystic ovary syndrome. Journal of Health, Wellness, and Community Research, 3(9). https://doi.org/10.61919/7w2kpm23

[24] Akhter, S., Chowdhury, N. M., Chowdhury, P., Barua, U., Parvin, A., Barua, L., and Yasmin, M. (2025). Evaluation of luteinizing hormone, follicle stimulating hormone and LH/FSH ratio in polycystic ovary syndrome of reproductive women. *Eastern Medical College Journal, 10*(1). https://doi.org/10.3329/emcj.v10i1.82516

[25] Xia, Q., Xie, L., Wu, Q., Cong, J., Ma, H., Li, J., Cai, W., and Wu, X. (2023). Elevated baseline LH/FSH ratio is associated with poor ovulatory response but better clinical pregnancy and live birth in Chinese women with PCOS after ovulation induction. *Heliyon, 9*(1), e13024. https://doi.org/10.1016/j.heliyon.2023.e13024

[26] Kizmaz, E., Coskun, M., Cerit, E. T., Alev, A., Akturk, M., Toruner, F., Karakoc, A., and Yalcin, M. M. (2025). Evaluation of thyroid hormone sensitivity in polycystic ovary syndrome. *Endocrine Abstracts, 110*, EP1332. https://doi.org/10.1530/endoabs.110.EP1332

[27] Palomba, S., Colombo, C., Busnelli, A., Caserta, D., and Vitale, G. (2023). Polycystic ovary syndrome and thyroid disorder: A comprehensive narrative review of the literature. *Frontiers in Endocrinology, 14*, 1251866. https://doi.org/10.3389/fendo.2023.1251866

[28] Kukreti, S., Jain, J. A., Jain, A., and Maurya, R. (2025). The relationship between gonadotropin level and thyroid dysfunction in the polycystic ovarian syndrome. *European Journal of Cardiovascular Medicine*, *15*(6), 626–629. https://doi.org/10.5083/ejcm/25-06-100

[29] Kaczmarczyk-Rot, P., Głuch, E., Sobol, M., Zegadło, A., Jurkiewicz, D., Niemczyk, S., and Leśniak, K. (2025). A novel method for assessing sinusitis activity in minimally symptomatic patients with ANCA-associated vasculitis: A pilot study. *Journal of Clinical Medicine, 14*(24), 8972. https://doi.org/10.3390/jcm14248972

[30] Ahmed, R. S., Aljabar, F. A. A., and Al-Shakir, N. M. M. (2025). Impact of insulin resistance in PCOS (LH/FSH) in a group of woman in Baghdad City. *Al-Esraa University College Journal for Medical Sciences*, *6*(9), Article 3. https://doi.org/10.70080/2790-7937.1045

[31] Li, Y., Wang, Y., Liu, H., Zhang, S., and Zhang, C. (2023). Association between HOMA-IR and ovarian sensitivity index in women with PCOS undergoing ART: A retrospective cohort study. *Frontiers in Endocrinology*, *14*, 1117996. https://doi.org/10.3389/fendo.2023.1117996

[32] Rambaran, N., and Islam, S. (2025). Decoding androgen excess in polycystic ovary syndrome: Roles of insulin resistance and other key intraovarian and systemic factors. *World Journal of Diabetes*, *16*(7), 108789. https://doi.org/10.4239/wjd.v16.i7.108789

[33] Abdelmonem, B. H., Abdelaal, N. M., Rashwan, E. K. E., Anwer, A. A., Hussein, R. M. A., et al. (2024). Decoding the role of CYP450 enzymes in metabolism and disease: A comprehensive review. *Biomedicines*, *12*(7), 1467. https://doi.org/10.3390/biomedicines12071467

[34] Wang, K., Li, Y., and Chen, Y. (2023). Androgen excess: A hallmark of polycystic ovary syndrome. Frontiers in Endocrinology, 14, 1273542. https://doi.org/10.3389/fendo.2023.1273542

[35] Zaboon, M., and Jassim, W. (2022). Detection of the effect of adiponectin and vitamin D on selected biochemical parameters in type 2 diabetes mellitus. *International Journal of Drug Delivery Technology*, *12*, 1748–1750. https://doi.org/10.25258/ijddt.12.4.42

[36] Joseph, T. S., Gillani, S. A., Shah, N., Ahmed, H. A., Aziz, A., and Zahra, S. K. (2025). Assessment of oxidative stress markers in women with PCOS versus controls: A cross-sectional study. *Journal of Health, Wellness, and Community Research*, *3*(16).

[37] Jahantigh, D., and Hosseinzadeh, A. (2017). XRCC5 VNTR, XRCC6 -61C>G, and XRCC7 6721G>T gene polymorphisms associated with male infertility risk: Evidences from case-control and in silico studies. *International Journal of Endocrinology*, *2017*, Article 4795076. https://doi.org/10.1155/2017/4795076

[38] Chai, Y., Hu, L., Zhu, Y., Quan, D., Li, Y., Wang, X., Yang, Y., and Wang, Z. (2025). Association of CYP3A5 rs15524 and CYP3A7 rs776744 polymorphisms on tacrolimus intrapatient variability and clinical outcomes in early post-heart transplantation. *BMC Medical Genomics*, *18*, 151. https://doi.org/10.1186/s12920-025-02231-3

[39] Alharazy, S., Naseer, M. I., Alissa, E. M., Robertson, D., Lanham-New, S., Alqahtani, M. H., and Chaudhary, A. G. (2021). Association of SNPs in GC and CYP2R1 with total and directly measured free 25-hydroxyvitamin D in multi-ethnic postmenopausal women in Saudi Arabia. *Saudi Journal of Biological Sciences*, *28*(8), 4626–4632. https://doi.org/10.1016/j.sjbs.2021.05.004