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STUDYING THE EFFECT OF GENETIC POLYMORPHISMS FOR CYP17A1 GENE ON WOMEN'S RISK OF POLYCYSTIC OVARIAN SYNDROME IN THE IRAQI GOVERNORATE OF DIYALA

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Abstract:

Polycystic ovary syndrome is one of the most common disorders affecting the reproductive and endocrine systems among women of reproductive age. Because several cytochrome P450 genes, such as CYP17A1, CYP19, and CYP1F, have genetic polymorphisms that are associated with an increased risk of developing PCOS, PCOS is a disease that is polygenic in origin. The goal of the current study is to investigate the association between the genetic polymorphism of the CYP17A1 gene in the exon segment at the location of rs6162 and rs6163 variations and the risk of developing disease. Venous blood was drawn from 24 samples of women with polycystic ovarian syndrome and 24 samples of healthy women between the ages of 20 and 40 at the Al-Batoul Teaching Hospital in the Diyala Governorate, Iraq. DNA sequencing findings revealed two variants, rs6162 and rs6163, in the 454 base pair exon segment of the CYP17A1 gene. The variation of rs6162 shows that the A allele and the GA genotype are more ubiquitous in such patient group and could be related with a greater risk of polycystic ovarian syndrome. For the variation of rs6163, it was revealed that the patient group had more frequencies of the A allele and the CA genotype than the control group. These results show the probability that such a genotype is a causal factor in the disease.

Keywords: PCOS , Exon , CYP17A1 , Polygenic.

Introduction

The hormonal balance related to ovaries in fertile females is highly affected by polycystic ovarian syndrome (Shams El-Din, 2010). It stands for a prevalent illness among reproductive-age women (Domestic et al., 2015). Five to ten women who are of reproductive age are affected by PCOS syndrome, which is characterized by a number of symptoms such as obesity, hirsutism, and anovulation (Legro et al., 2012). Small cysts containing ovarian fluid are a hallmark of PCOS, or polycystic ovary syndrome. They may be benign, non-cancerous tumors

inside the ovary that interfere with ovulation and make conception difficult (Al-Jabri et al., 2011; Sakban et al., 2014), or they may be benign, tiny tumors inside the ovary (Maria and Jacquse, 2015). Although PCOS is thought to be a chronic illness for which there is no cure, some of its symptoms can be managed by medication, lifestyle modifications, and infertility therapies (World Health Organization, 2023). It has been shown via the pathophysiology of the condition and several recent studies that women with the syndrome experience long-term detrimental consequences, such as psychiatric issues and sleep difficulties (Hung et al., 2014), Eighty percent of cases of reproductive infertility (Abeer et al., 2022) are linked to hypothalamic abnormalities and ovarian dysfunction (Pitchai et al., 2016), depressive disorders and abnormal eating behavior (Basheeruddin et al., 2020), anxiety about marriage, and childbearing expectations (Zehravi, 2021). Even though the ovary is the major source of malfunction, both the clinical indicators and the severity of symptoms are dependent on external circumstances. LH levels, insulin resistance (IR), and obesity (OB) are these variables. According to Tamirs Hugh (2012), the pathophysiology of PCOS syndrome involves four main disorders: increased and excessive androgen production, excessive insulin production, a defect in insulin function, and abnormal ovarian morphology with multiple cysts and increased ovarian size. Health issues such as type 2 diabetes, hypertension, high blood pressure, high cholesterol, heart disease, and endometrial cancer are more common in women with ovarian syndrome than in other women. The primary cause of infertility, polycystic ovarian syndrome, makes pregnancy challenging due to irregular menstruation. which lacks ovulation in addition (World Health Organization 2020). Many tiny fluid-filled cysts may develop in both ovaries of a woman with polycystic ovarian syndrome, and this condition may lead to pelvic inflammation (Lopez et al., 2014). Since polygenic origin is evident and specific genetic sites and genes linked to the disease have been identified, research has been conducted to determine the causes of polycystic ovary syndrome, which is inherited genetically through autosomal dominant genes (Nautiyal et al., 2022). Recent research has demonstrated that physiological mechanisms, the environment, and genes interact to cause conditions like insulin resistance, altered androgen secretion and action, gonad dysregulation, and chronic inflammation. The genetic basis for these conditions also plays a role (Azeez, 2020). According to certain research, functional mitochondrial disorders are thought to be a significant risk factor for PCOS (Zhang etal., 2019; Zeng etal., 2019). The amount of total testosterone in the serum positively correlates with changes in the number of mtDNA copies, and the quantity of mtDNA copies is a metabolic marker of mitochondria (Yang etal., 2020). Since the genes responsible for the syndrome have been identified, additional recent studies have demonstrated a connection between PCOS and other genetic factors, including stress factors, obesity, and physical inactivity, as well as a family history of the syndrome, particularly in first-degree relatives (CYP19A1, CYP11A1, CYP17A1). (HeidarZadah, etal. 2022; Parker and et al., 2022).

The current research aims to investigate the genetic polymorphisms of the CYP17A1 gene and their relationship to the risk of developing polycystic ovary syndrome in women.

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Martial and Methods

The current research was conducted in the Molecular Genetics Laboratory at the College of Education for Pure Sciences at the University of Diyala in Iraq. Samples from polycystic ovarian syndrome patients who visited Al-Batoul Teaching Hospital, as well as samples from healthy women in Diyala Governorate, were used in the study, between October 2022 and May 2023. Venous blood samples were obtained from women with polycystic ovarian syndrome as well as from healthy women. There were 48 research samples in all; 24 were from women in good health, and the other 24 were from women who had polycystic ovarian syndrome. DNA was extracted using the System gDNA Miniprep Blood ReliaPr extraction kit, which was provided by Bioneer in South Korea. To amplify the CYP17A1 gene in exon region at the variant site rs6162 using the forward specific primer (3- CTGGAAGCCCCATTCTAGGC -5) , reverse specific primer (3- TGTGCCCTAGAGTTGCCACA -5) and in the exon region at the variant site rs6163 using the forward specific primer (3- GTGGCGGAGGTAATCAGGAA -5) , reverse specific primer (3- GAGGTGTAAGGGCAAGAGTGG -5). 1.5 µl of forward primer, 1.5 µl of reverse primer, 3 µl of DNA, 5 µl of master mix, and 14 µl of free nuclease water constituents of the PCR mixture. The total volume of the reaction product for each sample was 25 µl. Next, the polymerase chain reaction device's samples of healthy women and patients with polycystic ovarian syndrome were mixed with the reaction mixture. The following reaction conditions were programmed into the apparatus: five minutes at 94°C for initial denaturation, thirty seconds at 94°C for denaturation, thirty seconds at 63°C for primer annealing, five minutes at 72°C for extension, and five minutes at 72°C for final extension. This was carried out due to a total of 35 cycles involving primer annealing, denaturation, and extension. After completing the PCR gene amplification operation, the samples were electrophoresed on a 1% agarose gel for 1.5 hours at 90 V. Afterward, the product of amplification was directed to Macrogen Company in South Korea so that the CYP17A1 gene could be nucleotide sequenced by employing the Sanger method. According to tests of nucleotide sequence statistics adopting the Genius application, the Hardy-Weinberg equation was employed to detect which genotype is a defensive factor and which genotype is a causative one. (Alzubadiy et al., 2019).

Results

Amplification of the CYP17A1 gene's exon area, that consists of the variations rs6162 and re6163, taking place in all polycystic ovarian syndrome patients and sound individuals.

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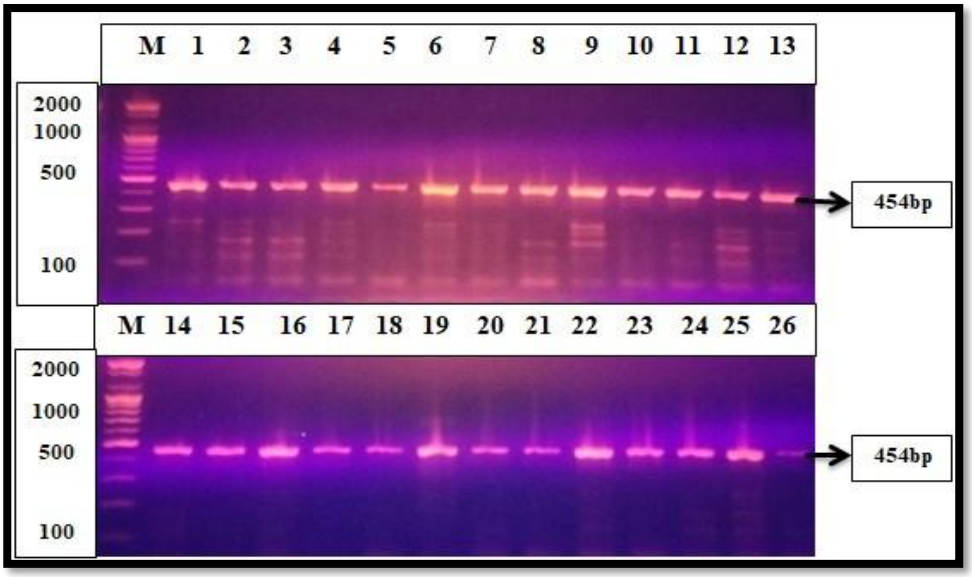

Fig 1. illustrates the result of amplification of the CYP17A1 exon segment, that has the variants rs6163 and rs6162, in women from the community of Diyala city having polycystic ovarian syndrome and sound people. The sample was migrated on a 1.5% agarose gel for 1.5 hours at an electrical potential of 90 volts, stained with ethidium bromide dye, and captured on camera under ultraviolet light. Number from 1 to 13 denote sick samples, while Number from 14 to 26 denote healthy controls.

The amplification of the CYP17A1 gene's exon region at the location of the rs6163 and rs6162 variations is seen in Figure 1. According to the amplification results, the resultant bands for both the polycystic ovary syndrome patient and healthy woman samples, as well as for both variations, had a molecular weight of 454 base pairs.

Nucleotide sequence comparison between the GenBank sample and the present study samples at the location of the rs6162 and rs6163 variations in the exon region of the CYP17A1 gene.

The presence of a point mutation of the transition type at position 318 of the nucleotide sequence of the gene for the variant rs6162, as shown in Figure 2, and a point mutation of the transversion type at position 470 of the nucleotide sequence of the gene were revealed when the nitrogenous bases of the exon segment of the CYP17A1 gene were aligned with the reference sequence in GenBank. similar to Figure 3.

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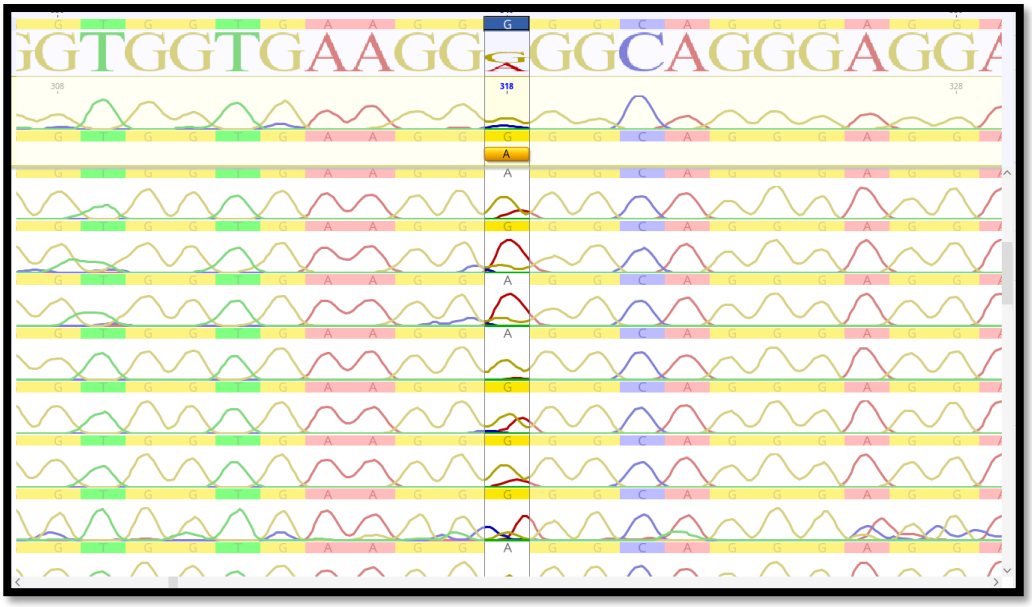

Figure 2. shows a comparison of the nitrogenous bases of a portion of the CYP17A1 gene between samples from patients with polycystic ovarian syndrome and healthy women, as well as a comparison with the GenBank sample that displays the type and position of the rs6162 G/A variation.

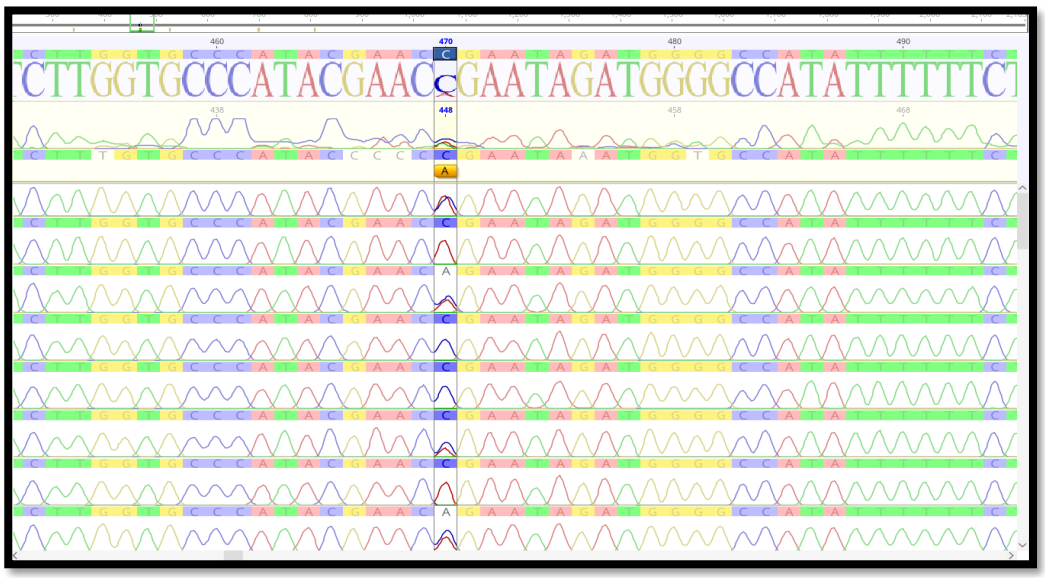

Figure 3. shows a comparison of the nitrogenous bases of a portion of the CYP17A1 gene between samples from patients with polycystic ovarian syndrome and healthy women, as well as a comparison with the GenBank sample that displays the type and position of the rs6163 C/A/T variation.

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Genotypes of CYP17A1 gene in exon region at rs6162 variant site in healthy and polycystic ovary syndrome women

Table 1 results show that the CYP17A1 gene contains three genotypes: GG, GA, and AA. These genotypes are important to consider when assessing the relationship between CYP17A1 genotypes and alleles in the exon region at the rs6162 G/A heterozygous location in PCOS patients and healthy controls. Fisher's probability values of 0.773, 0.569, and 0.759, respectively, supported the statistical analysis that found no statistically significant differences between PCOS patients and healthy women.

Table 1. Genotype distribution and allele frequency of CYP17A1 rs6162 G/A SNPs

Genotype // rs6162 G/A	Patients No. (%)	Control No. (%)	Fisher's/P-value	O.R. (C.I.)
GG	8(33.33%)	9 (37.5%)	0.773 NS	0.83(0.25 - 2.80)
GA	10(41.66%)	8(33.33%)	0.569 NS	1.43(0.43 - 4.77)
AA	6(25%)	7(29.16%)	0.759 NS	0.81(0.21 - 3.01)
Total	24 (100%)	24 (100%)		
Allele Frequency				
G	26 (54.17%)		26 (54.17%)	O.R. (C.I.)= 1.00 (0.45 - 2.23)
A	22 (45.83%)		22 (45.83%)	O.R. (C.I.) = 1.00 (0.44 - 2.25)
NS: Non-Significant.				

The distribution of allelic frequencies and genotypes of the CYP17A1 gene in the exon region at the rs6162 variant location has been studied between PCOS patients and healthy controls in accordance with the Hardy-Weinberg law. Based on the Hardy probability values reaching 0.4307and 0.1074, respectively, the results in Table 2 show that there are no statistically significant differences between the observed and expected values for the group of polycystic ovary syndrome patients and healthy women.

Table 2. Hardy-Weinberg equilibrium-based predicted genotype and allele frequencies of the CYP17A1 gene's exon at the rs6162 G/A variant location

Hardy P-values	Allele frequencies		Genotype // rs6162 G/A					Groupe
0.4307 NS	A	G	AA	GA	GG	No.	Observed	Patient 24
	22	26	6	10	8			
	45.83	54.17	25	41.66	33.33	%		
	Not diagnosed		5.04	11.92	7.04	No.	Expected	
			21.01	49.65	29.34	%		
0.1074 NS	22	26	7	8	9	No.	Observed	Contro 24
	45.83	54.17	29.16	33.33	37.5	%		
	Not diagnosed		5.04	11.92	7.04	No.	Expected	
			21.01	49.65	29.34	%		

NS: Non-Significant.

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Genotypes of CYP17A1 gene in the exon region at rs6163 C/A/T variant site in healthy and polycystic ovary syndrome women

Table 3 results show that the CYP17A1 gene contains three genotypes: CC, CA, and AA. These genotypes are important to consider when assessing the relationship between CYP17A1 genotypes and alleles in the exon region at the rs6163 C/A/T variant location in PCOS patients and healthy controls. Fisher's probability values of 0.559, 0.266, and 0.505, respectively, supported the statistical analysis that found no statistically significant differences between PCOS patients and healthy women.

Table 3. Genotype distribution and allele frequency of CYP17A1 rs6163 C/A/T SNPs

Genotype // rs6163 C/A/T	Patients No. (%)	Control No. (%)	Fisher's/P-value	O.R. (C.I.)
CC	7 (29.16%)	9 (37.5%)	0.559 NS	0.69 (0.20 - 2.36)
CA	13 (54.17%)	9 (37.5%)	0.266NS	1.97 (0.61 - 6.40)
AA	4 (16.66%)	6 (25%)	0.505 NS	0.60 (0.13 - 2.57)
Total	24 (100%)	24 (100%)		
Allele Frequency				
C	27 (56.25%)	27 (56.25%)	O.R. (C.I.) = 1.00 (0.44 2.26)	
A	21(43.75%)	21(43.75%)	O.R. (C.I.) = 1.00 (0.44 - 2.26)	
NS: Non-Significant.				

The distribution of allelic frequencies and genotypes of the CYP17A1 gene in the exon region at the rs6163 C/A/T variant location has been studied between PCOS patients and healthy controls in accordance with the Hardy-Weinberg law. Based on the Hardy probability values reaching 0.6224and 0.2434, respectively, the results in Table 4 show that there are no statistically significant differences between the observed and expected values for the group of polycystic ovary syndrome patients and healthy women.

Table 4. Hardy-Weinberg equilibrium-based predicted genotype and allele frequencies of the CYP17A1 gene's exon region at rs6163 C/A/T variant location

Hardy P-values	Allele frequencies		Genotype // rs6163 C/A/T					Groupe
0.6224 NS	A	C	AA	CA	CC	No.	Observed	Patients 24
	21	27	4	13	7			
	43.75	56.25	16.66	54.17	29.16	%		
	Not diagnosed		4.59	11.81	7.59	No.	Expected	
			19.14	49.22	31.64	%		
0.2434 NS	21	27	6	9	9	No.	Observed	Control 24
	43.75	56.25	25	37.5	37.5	%		
	Not diagnosed		4.59	11.81	7.59	No.	Expected	
			19.14	49.22	31.64	%		

NS: Non-Significant

Discussion

In order to investigate variations within the exon segment of the CYP17A1 gene located on the tenth chromosome, Figures 2 and 3 compare the sequences of all 48 samples from the current study, which included two groups: the first group, consisting of 24 samples from women with polycystic ovary syndrome, and the second group, consisting of 24 samples from healthy women, with each other in a chart. When comparing the nucleotide sequence of the CYP17A1 gene from patients with polycystic ovarian syndrome and healthy women with the reference DNA sequence, the results of amplification of the CYP17A1 exon region revealed the presence of a point mutation of the transition type within the rs6162 variant and a point mutation of the transversion type within the variant rs6163.

The results in Table 1 when comparing polycystic ovary syndrome patients with healthy women showed that the observed number of patients carrying the homozygous genotype GG was 8 and the G allele was 26. A decrease was recorded in the group of polycystic ovary syndrome patients, and the percentage was calculated at 33.33 and 54.17, respectively, compared to the healthy group, as it reached 9 and a percentage of 937.5 and 26 and a percentage of 54.17, respectively. According to Fisher's probability P = 0.569, there are no significant differences between the group of patients and the group of healthy women. Therefore, the homozygous genotype GG is considered a protective factor for the disease according to the value of the odds ratio was 0.83. Additionally, the data demonstrated that the proportion of female patients with variant genotype 10 (GA) and the A allele (22) increased in the patient group, reaching 41.66 and 45.83, respectively, in comparison to the control group, which recorded 8 (33.33) and 22 (45.83). Fisher's probability was determined to be P = 0.569, indicating that there are no statistically significant differences between the patient and healthy groups. Therefore, the odds ratio values, which reached 1.43 and 1.00, respectively, suggest that the GA genotype is a causative factor for the disease. While the homozygous genotype AA and the A allele recorded a significant decrease in female patients, reaching 6 by 25 and 22 by 45.83, respectively, compared to healthy women, which reached 7 by 29.16 and 22 by 45.83. According to Fisher's probability P = 0.759, there are no significant differences between healthy patients, so the genotype AA is a protective factor for the disease, and the odds ratio value is 0.81.

According to the Hardy-Weinberg law, the three genotypes—AA, GA, and GG—at the variant site rs6162 G/A in the group of polycystic ovary syndrome patients and healthy females are balanced, as demonstrated by Table 2's results. The Hardy probability values for the three genotypes and alleles reached 0.4307 and 0.7074 in the group of polycystic ovary syndrome patients, respectively, indicating that there are no statistically significant differences between the observed and expected values for the three genotypes and alleles. In this context, a study was carried out by researcher Resgoun, etal. (2023) to assess the genetic variants of the CYP17A1 gene in individuals with diabetes. Researchers discovered that those with the homozygous genotype TT at variant site rs17115149 have increased levels of blood sugar, arteriosclerosis, and weight gain in comparison to those without the genotype. CC and TC, as the odds ratio value for the T allele vs. the C allele was [OR = 1.34, CI 1.020 -1.849], indicating

that the TT genotype is linked to the risk of TDM2 and atherosclerosis. Accordingly, a study by the researcher (Eric et al. 2012) revealed a substantial correlation between the steroid levels in blood plasma and the genetic polymorphism of the CYP17A1 gene at the rs6162 variant site, with a 20% difference in DHEA-S levels.

Table 3 showed that there were 7 and 27 polycystic ovary syndrome patients with homozygous CC genotypes and C alleles, respectively. Fisher's probability P = 0.559 indicates that there are no significant differences between the patient and healthy woman groups, indicating that the CC genotype is a protective factor for the disease according to the value of the odd ratio of 0.69. The group of polycystic ovary syndrome patients showed a significant decrease, with ratios of 29.16 and 56.25, respectively, compared to the group of healthy women, reaching 9 with a ratio of 37.5 and 27 by 56.25, respectively. Since there are no significant differences between female patients and healthy individuals, the variant CA genotype and the A allele increased noticeably in female patients, reaching 13 by 54.17 and 21 by 43.75, respectively, according to Fisher's probability = 0.266. Therefore, the CA genotype is thought to be a causative factor for the disease, as indicated by the values of the odds ratio of 1.97 and 1.00, respectively. The results indicated that there were 4 female patients with the homozygous genotype AA and 21 individuals with the A allele. According to Fisher's probability P = 0.505, there was a significant drop in the female patients, with the ratios being 16.66 and 43.75, respectively, compared to the healthy group, which reached 6 by 25 and 21 by 43.45, respectively. Given that there are no appreciable variations between female patients and healthy female, the AA genotype is thought to be a protective factor against the illness, with an odds ratio of 0.60.

Results shown in Table 4 demonstrated that, in accordance with the Hardy-Weinberg law, the distribution of the three genotypes—CC, AA, and CA—as well as the allelic frequency of the CYP17A1 gene at the heterozygous site rs6163 C/A in the study population are balanced. This is because there are no statistically significant differences between the observed and expected values for any of the three genotypes or alleles. In the group of suffering females and the group of sound ones, the probability values of Hardy were 0.6224 and 0.2434 respectively. Contextually, the CYP17A1 gene's genetic polymorphism at the rs6163 variant location was detected by Al-Rubaei et al. (2017) in females with polycystic ovarian syndrome. It was found out that females having polycystic ovarian syndrome, the CC genotype was associated to high blood levels of progesterone and estrogen throughout the premenopausal phase. Furthermore, the odds percentage value rose to [OR: 1.4, CI: 1.03–1.08] for endometrial infections. Moreover, a research by Robles-Fernandez et al. (2017) found out that four variants of the CYP17A1 gene, rs6163, rs6162, rs743575 and rs1004467, affect the evolution of prostate cancer and, consequently, reduce the production of androgens, which in turn causes the process of castration in cancer resistance following chemotherapy. Dehydroepianderone (DHEA), a steroid hormone that functions as a precursor to androgen production inside the tumor, that adjusts cancer growth, is also associated to high risk of cancer.

Conclusion

It was concluded that GA and CA variants of the rs6162 and rs6163 variants in women, respectively, genetic polymorphisms of the CYP17A1 gene in the exon area at the variant locations were related with the possibility of developing polycystic ovarian syndrome.

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