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ASSOCIATION OF CYP17A1 GENE & CYP19A1 GENES POLYMORPHISMS FOR POLYCYSTIC OVARIAN SYNDROME (PCOS) IN LOCAL FEMALE POPULATION OF BAHAWALPUR

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ABSTRACT

Background: *Polycystic Ovarian Syndrome (PCOS) is a multifactorial endocrine condition that leads to hyper-androgenism, menstrual irregularities, and numerous cysts which eventually result in infertility. PCOS could be associated with social environmental, hormonal, and genetic factors.* ***Study Objective:*** *This study aimed to investigate the genetic association of SNP rs2414096 in the CYP19A1 and rs743572 in the CYP17A1 genes with PCOS in the local female population of District Bahawalpur.* ***Method:*** *For this case-control association study, 100 patients were ascertained with PCOS, and 50 healthy individuals as controls were enrolled. Furthermore, factors like BMI, and Ferriman–Gallwey (FG) score were also explored. Blood samples of all the participants were drawn, and DNA was extracted and analyzed for polymorphisms of CYP19A1 and CYP17A1 genes by using the tetra ARMS-PCR method. A p-value of less than 0.05 was considered to be significant.* ***Results:*** *There was a significant association between hirsutism, and body mass index (BMI), with PCOS. The genotypic distribution for CYP17A1 showed that the wild-type frequency is significantly higher within PCOS females (68%) and controls (70%) with an odd ratio of 1.068 (Cl: 0.57-1.98). The genotypic distribution for CYP19A1 revealed a high frequency of wild type in patients (67%) and also in the control group (86%) with an odd ratio of 2.625 (Cl: 1.117-6.167).* ***Conclusion:*** *There was no significant difference in the genotypic distribution of CYP17A1 alleles and CYP19A1 alleles between patients and controls. This suggested that CYP17A1 and CYP19A1 polymorphisms are not significantly associated with PCOS in the Bahawalpur population.*

Keywords: *CYP17A1; CYP19A1; Ferriman–Gallwey; Hirsutism; Menstrual irregularity; Polycystic Ovarian Syndrome; Polymorphism.*

INTRODUCTION

Poly Cystic Ovarian Syndrome (PCOS) is the most prevalent hormonal imbalance disorder in adult females leading to primary infertility with anovulation. The prevalence of PCOS is difficult to define due to limited data but according to estimates, this syndrome is becoming very common among women with a prevalence between 2.2% to 26% [1]. Even though the exact reason is yet unknown, PCOS is a complex condition with genetic, metabolic, endocrine, and environmental problems, emerging as a multifactorial hormonal disorder [2]. The main factors involved in PCOS pathogenesis are excess production of insulin in the body, excess androgen synthesis in the ovary, and low-grade inflammation that can cause heart problems. PCOS is not a life-threatening syndrome but it may affect women's health in many ways. These health problems include abnormal uterine bleeding which can lead to heavy blood loss in women, infertility, stress or depression due to unwanted hairs and acne, mood, sleep apnea, eating disorders, type 2 diabetes mellitus, endometrial cancer, liver inflammation [3], and cardiovascular disorders [4]. Also miscarriages, premature birth, and preterm labor are common in women suffering from PCOS [5,6]. The term polycystic ovarian syndrome illustrates the presence of several small cysts in the ovaries. Cyst formation is not the only symptom of PCOS, because many women with this syndrome do not have cysts and vice versa [7]. Cysts are formed in ovaries due to elevated androgen levels, inhibiting follicle development and resulting in dysmenorrhea [8].

In mature females, this disease is triggered by hormonal imbalance in their reproductive age between ages 12 to 51 consequence in multiple cysts in the female gonads, oligomenorrhea (irregular menstruation), amenorrhea (absence of menstruation for more than three months), acne, hair loss, obesity, darkened skin patches around the neck or under breasts, trouble losing weight, and hirsutism. Adult females affected by PCOS are more prone to experience sleep apnea, depression, and anxiety [9] and might be linked to a great risk of pre-natal problems like gestational diabetes during pregnancy, preeclampsia, and miscarriages, thus implicating defects in the steroid synthesis pathway. Studies revealed that these clinical parameters are linked with susceptibility to PCOS [10]. Many genes like CYP17, CYP11, and CYP19 are reported to perform important roles in the progression of hyperandrogenism in PCOS [11].

Cytochrome P450, family 19, subfamily A, polypeptide 1 (CYP19A1) is found on the long arm of chromosome 15 (15q21.1), extents 123 kilobytes, and has ten exons [12]. CYP19A1 encodes the aromatase p450 enzyme which is considered an important component for the synthesis of estrogen from androgens [13]. In obese women suffering from PCOS, aromatase activity is reduced and may be further decreased due to the development of hyperandrogenemia [14]. The suppressed expression of aromatase was because of the production of protein in small amounts from reduced quantity of CYP19A1 mRNA [15]. A monooxygenase protein is also encoded by CYP19A1 and performs an important function in the production of lipids, cholesterol, and steroids. It is found in the endoplasmic reticulum and plays a crucial function in the estrogen production pathway in gonadal and extra-gonadal tissues. Due to a mutation in the CYP19A1 gene, the risk of PCOS progression in adult females is doubled [9].

CYP17A1 is positioned at chromosome 10q24.32 [16]. The CYP17A1 gene is encoded for the enzyme P450c17α. These are the enzymes of the endoplasmic reticulum that control steroid production, cholesterol metabolism, and drug metabolism reactions. The expression of the CYP17A1 gene is observed in many tissues, including the gonads and adrenal cortex, any defect in this gene may cause functional abnormalities. High levels of androgen in PCOS patients with functional hyperandrogenism may result from increased P450c17 enzyme activity [17]. An additional transcription factor binding site (Sp1) that is involved in the regulation of the expression of CYP17 and control androgen levels are created at −34 position (−34 T/C) in the 5'UTR the promoter region of CYP17A1 resulting in polymorphism [18]. The present study was designed to examine the association of the CYP19A1 and CYP17A1 gene polymorphisms with the progression of PCOS along with the clinical characterization of this disease The main objectives of this research are to determine the correlation between demographic parameters and PCOS pathogenesis along with the association of mutations in the CYP17A1 and CYP19A1 genes with the development of Polycystic Ovarian Syndrome.

Materials and Methods

Subjects

In this case-control study, one hundred women visiting the Department of Obstetrics & Gynecology, Bahawal Victoria Hospital, Bahawalpur, diagnosed with PCOS, and fifty healthy volunteers of the same age range between 13 and 40 years with their informed consent form were enrolled from Bahawalpur, Pakistan. This study was permitted by the Institutional Review Board at Govt. Sadiq College Women's University, Bahawalpur.

Inclusion and Exclusion Criteria

The PCOS diagnosis was done by using Rotterdam criteria based on three abnormalities [19]. In this criteria, any two abnormalities were included for diagnosis: 1) ovarian dysfunction (oligo-ovulation having menstrual cycle longer than 35 days or anovulation means no menstrual cycle), (2) Polycystic ovaries by ultrasound, and (3) hyperandrogenism. Age-matched healthy females who had regular periods and showed no clinical signs of hirsutism, obesity, thyroid, infertility, or any other chronic condition were enrolled as the control for the study. The women with hyperprolactinemia, Cushing syndrome, androgen-producing tumors, and congenital adrenal hyperplasia were excluded from the study.

Clinical Analysis

Age, weight (w), height (h), body mass index (BMI), ethnicity, hirsutism, acne, family diabetes history, and family reproductive history was filled out by the participants of the study on a questionnaire to get all the required information. The presence of cysts, hormonal imbalance, and profile was checked by transabdominal ultrasound that is diagnosed by a gynecologist. BMI was calculated by formula (BMI = weight (kg)/ Height^2 (m^2)). Hirsutism was measured by the Ferriman-Gallwey (FG) score in which a 0 (no terminal hairs) to 4 (various terminal hairs) score was given based on the presence of hairs in 9 different locations [20]. Three milliliters of blood were drawn by venipuncture from each subject. To avoid blood clotting, blood was stored in EDTA vials and preserved at 20°C for later use.

Genotyping

Genomic DNA was extracted by using a DNA isolation Kit (WizPrep™ gDNA Mini Kit) (Cell/Tissue). DNA amplification was done by Tetra-Amplification Refractory Mutation System-Polymerase Chain Reaction) (ARMS-PCR), technique. In this technique, four primers are used to detect the single nucleotide polymorphism (SNP). The PCR product was electrophoresed on 1.2% (w/v) agarose gel with ethidium bromide and a gel documentation system was used to record the results of that gel. For amplification of SNP (c.-34T>C) of CYP17A1 gene, a volume of 20µl reaction mixture was used containing 2µl genomic DNA, 10.5µl PCR water (Rnase or Dnase free water), 0.6µl Taq polymerase, and 2µl Taq buffer (2.5mM), 2µl dNTPs and 0.5µl of each outer primer for CYP17A1 (c.-34T>C) (Outer Forward: 5' AGATGGGCACCACTTACCATTTGA 3', Outer Reverse: 5'ACTCTGGAGTCATTCAAGCATGGG 3') and 1.0µl of each inner primer (Inner Forward: 5'CGGCAGGCAAGATAGACATCG 3'), Inner Reverse: 5' TGCCACAGCTCTTCTACTCCCCT 3') and for CYP19A1: (Outer Forward: 5' GTGTGCTAATTTCTTCCCAGGTTA 3', Outer Reverse: 5' TTCTCTGATATAAGCAGCACCAAA 3') and 1.0µl of each inner primer (Inner Forward: 5' CTTTTGTTACCCTCAAAAAAGACTACA 3', Inner Reverse: 5' AGATTTAGCTTAAGAGCCTTTTCTTACAC 3') were used. The thermal cycler conditions were the following; initial denaturation at 94°C for 5 min, 94°C for 45 seconds, annealing at 65°C for 1 min, extension at 72°C for 45 secs, followed by a final extension at 72°C for 5 min. The PCR products were observed by 1.2% agarose gel. In the case of CYP17A1 heterozygous genotype (CT), three bands of 569bp, 321bp, and 292bp were seen. Samples with homozygous wild (TT) genotype have two bands of 569bp and 321bp, while samples with homozygous mutant (CC) showed bands of 569bp and 292bp [21]. Samples with CYP19A1 homozygous wild (GG) genotype have two bands of 520bp and 320bp, while samples with homozygous mutant (AA) showed bands of 520bp and 256bp. The heterozygous genotype (GA) shows three bands of sizes 520bp, 320bp, and 256bp [21].

Statistical Analysis

IBM SPSS Statistics Version 20 was used for statistical analysis. Cross-tabulation was used to check the prevalence of clinical parameters of PCOS among both groups. The Independent t-test was applied to compare continuous variables and a chi-square test was used to compare categorical variables. The logistic regression analysis was used to calculate the odds ratio (OR) and significance value of different demographic parameters. The genotypic and allelic frequencies were examined using $\chi 2$, OR, and 95% CI. A P-value of <0.05 was considered statistically significant.

Results

Impact of Anthropometric and Demographic Factors on the Phenotype

The frequencies of all variables among controls and PCOS cases were calculated. Patients and controls of age between 20 to 40 years were selected and divided into four groups. Analysis of anthropometric and demographic factors revealed, that 29% of patients were under the age of 20 years, while the age range of 21 to 30 years had the largest frequency of PCOS patients (53%), this data indicates that females with the age group of 21-30 years were remarkably more prone to PCOS. Comparison of obesity showed that a significantly (78%; P< 0.001) higher number of PCOS patients were obese. Since 78% (P< 0.001) of the surveyed women with PCOS had acne and 79% (P< 0.001) had hirsutism. Furthermore, it was observed that menstrual cycle irregularity had a significant impact on the prevalence of PCOS as 91% had abnormal menstrual cycles, in addition, 90% of PCOS patients had oily skin versus the control group. However, a little number of surveyed individuals showed a family history of PCOS (Table 1; Figure 1).

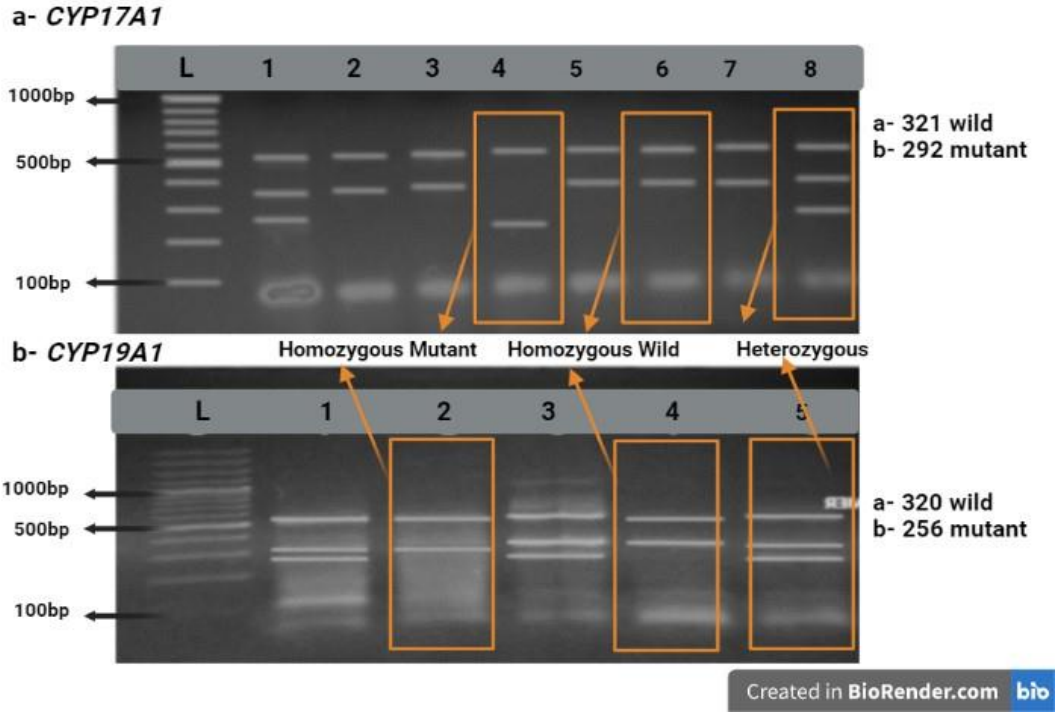

Figure 1: a). 1.2% gel was loaded with PCR product. Lanes 2,3,5,6, and 7 contain wild-type genotypes, lane 1 shows a heterozygous genotype and 4 is homozygous. b) Lanes 1, 3, and 5 demonstrate heterozygous genotype (256 bp) and lane 2 and 4 suggests wild type (320 bp).

Table 1: Association of Risk Factors (Age, Obesity, Family History, Acne, Hirsutism, Menses, Oily skin, and polycystic ovaries) with Polycystic Ovarian Syndrome in patients (n:100) and controls (50)

Parameters		PCOS (n=100)	Controls (n=50)	Total (n=150)	p-value
Age	<20	29 (29%)	17 (34%)	46	0.019**
	21-30	53 (53%)	19 (38%)	72	
	31-40	18 (18%)	10 (20%)	28	
	>40	0 (0%)	4 (8%)	4	
Obesity	BMI > 24.9	78 (78%)	6 (12%)	84	< 0.001*
	BMI < 24.9	22 (22%)	44 (88%)	66	
Family History	Seen	31 (31%)	14 (28%)	45	0.85
	Not seen	69 (69%)	36 (72%)	105	
Acne	Present	78 (78%)	6 (12%)	84	< 0.001*
	Absent	22 (22%)	44 (44%)	66	
Hirsutism	Present	79 (79%)	6 (12%)	85	< 0.001*
	Absent	21 (21%)	44 (44%)	65	
Menses	Irregular	91 (91%)	9 (18%)	100	< 0.001*
	Regular	9 (9%)	41 (82%)	5	
Oily Skin	Present	90 (90%)	33 (66%)	123	< 0.001*
	Absent	10 (10%)	17 (34%)	27	
Polycystic Ovaries	Present	65 (65%)	7 (14%)	72	< 0.001*
	Absent	35 (35%)	43 (86%)	78	

. *p ≤0.05,**p ≤ 0.01,***p≤ 0.001, p^{ns}=non-significant

The mean $^{\pm}$ Standard deviation for demographic variables such as individual's age, height, weight, FG score, and Body Mass Index (BMI) were measured to investigate their significance in diseased people versus healthy controls and a noticeable difference was observed in weight, BMI, and FG Score values between cases and controls (Table 2).

Table 2: Comparison of Mean±SD of Demographic Variables (Age, Weight, Height, BMI, and FG score) between PCOS and Control Groups

Parameters	PCOS (n=100)	Controls (n=50)	p-value
Age	24.29 ± 6.41	25.64 ± 8.69	0.284
Weight	65.37 ± 10.99	53.70 ± 7.56	< 0.001*
Height	1.56 ± 0.08	1.58 ± 0.07	0.114
BMI	26.83 ± 4.31	21.57 ± 3.83	< 0.001*
FG Score	13.49 ± 5.36	4.34 ± 3.34	< 0.001*

*p-value less than 0.05 is significant, suggesting that the observed differences are unlikely to occur due to random chance alone. PCOS: polycystic ovarian syndrome, BMI: body mass index, FG score: Ferriman-Gallwey score.

Association of demographic factors like age, BMI, acne, hirsutism, dysmenorrhea, and family history with polymorphisms was checked and it was depicted that none of the demographic factors of PCOS have a significant association with polymorphism of CYP17A1 but some factors like hirsutism, oily skin, and polycystic ovaries have a significant association with polymorphism of CYP19A1 (Table 3).

Table 3: Association of Demographic Factors (Obesity, Family History, Acne, Hirsutism, Menses, Oily skin, and polycystic ovaries) with CYP17A1 and CYP19A1 Polymorphisms

Parameters		CYP17A1					CYP19A1			
		T/T	T/C	C/C	Total	p-value	G/G	G/A	Total	p-value
Obesity	BMI > 24.9	45	16	5	66	0.761	52	17	69	0.6
	BMI < 24.9	58	22	4	84		58	23	81	
Family History	Seen	67	31	7	105	0.143	31	14	45	0.4
	Not seen	36	7	2	45		79	26	105	
Acne	Present	42	20	4	66	0.453	52	13	65	0.1
	Absent	61	18	5	84		58	27	85	
Hirsutism	Present	44	17	4	65	0.975	29	11	40	0.01*
	Absent	59	21	5	85		56	54	110	
Menses	Irregular	37	10	3	50	0.962	30	10	40	0.1
	Regular	66	28	6	100		67	43	110	
Oily Skin	Absent	21	5	1	27	0.524	46	9	55	0.03*
	Present	82	33	8	123		64	31	95	
Polycystic Ovaries	Present	52	22	4	78	0.661	64	16	80	0.04*
	Absent	51	16	5	72		46	24	70	

*p ≤0.05,**p ≤ 0.01,***p≤ 0.001, p^{ns}=non-significant. CYP17A1: Genotypic distribution of the CYP17A1 polymorphism (T/T, T/C, & C/C) and CYP19A1 polymorphism (G/G, G/A & A/A).

The logistic regression analysis was also applied to several binary (family history, acne, hirsutism, monthly irregularities, oily skin, and ovarian polycysts) and continuous (age and BMI) variables. A variable increases the risk of prognosis when the odd ratio is larger than 1. So, patients aged 21 to 30 were more likely to have PCOS (2.920 (0.807-10.56) as compared to the age group of 11 to 20, (1.350; 95%CI: 0.494-3.684), and both are more prone to develop the disease as compared to the women aged above 40. Patients with obesity (24.9) had a 2.370 (95%CI: 0.037-154.77) times higher chance of PCOS progression than those with a BMI under 24.9. Females with a family history of PCOS had a higher chance to develop PCOS than those without a family history. The chances of PCOS in women were also increased in cases of acne, hirsutism, oily skin, ovarian polycysts, and irregular menstruation (Table 4).

Table Error! No text of specified style in document.**: Logistic Regression Analyses of Different Parameters Associated with Polycystic Ovarian Syndrome with 95%CI**

Parameters		PCOS (n=100)	Controls (n=50)	Odds ratio (95%CI)	p-value
Age	11-20	29 (29%)	17 (17%)	1.350 (0.494-3.684)	0.433
	21-30	53 (53%)	19 (19%)	2.920 (0.807-10.56)	0.558
	31-40	18 (18%)	10 (10%)	-	0.103
	>40	0 (0%)	4 (4%)	1.00 (ref)	0.999
Obesity	BMI > 24.9	78 (78%)	6 (12%)	2.370 (0.037-154.77)	< 0.001*
	BMI < 24.9	22 (22%)	44 (88%)	1.00 (ref)	
Family History	Seen	69(69%)	36 (72%)	43.3(0.38-4942)	0.85
	Not seen	31 (31%)	14 (28%)	1.00 (ref)	0.119
Acne	Present	22 (22%)	44 (44%)	0.003(0-0.414)	0.02*
	Absent	78 (78%)	6 (12%)	1.00 (ref)	
Hirsutism	Present	21 (21%)	44 (44%)	0.004(0-0.255)	0.009***
	Absent	79 (79%)	6 (12%)	1.00 (ref)	
Menses	Irregular	9 (9%)	41 (82%)	0.007(0-0.536)	0.025*
	Regular	91 (91%)	9 (18%)	1.00 (ref)	

Oily Skin	Present	10 (10%)	17 (34%)	4.5(0.104-195)	0.433
	Absent	90 (90%)	33 (66%)	1.00 (ref)	
PCOM*	Present	35 (35%)	43 (86%)	0.022(0-0.448)	0.074
	Absent	65 (65%)	7 (14%)	1.00 (ref)	

PCO polycystic ovarian morphology. OR (95% CI): Presents the odds ratio (OR) and its corresponding 95% confidence interval, providing information on the strength of the association between the genotype/allele and PCOS.

The genotypic and allelic frequencies of CYP17A1 with the corresponding odds ratio (OR), 95% confidence interval (CI), and significant association (χ^2, p-value) are summarized in Table 5. Among the PCOS group, 68% were wild homozygous (T/T), 6% polymorphic homozygous (C/C), and 26% were heterozygous (T/C). Among controls, 35% and 12% of individuals were wild homozygous and heterozygous genotypes, respectively. The homozygous variant was 6% in the control population. There is no significant difference between the distribution of polymorphic allele (C) and wild-type allele (T) of the homozygous and heterozygous group between cases and controls with $\chi^2 = 0.072$ and $p = 0.96$. In some cases, 81% and 19% had T allele and C allele, respectively. 82% of the T allele and 18% of the C allele were present in the control group with non-significant values (1.068 OR; 0.574 - 1.987 95% CI; $\chi^2 = 0.043$ and $p = 0.834$. For CYP19A1 mutation, the distribution of mutant allele (A) and wild-type allele (G) is not significantly different in PCOS patients and healthy people with (OR: 2.625; CI: 1.117-6.167; $\chi^2 = 0.773$ and $p = 0.208$). The ratio of wild type G allele was 83.5% and 93% in cases and controls correspondingly but the percentage was 16.5% and 7% for mutant type A allele in both diseased and normal people (Table 5).

Table 5.: Genotype and Allelic Frequencies of CYP17A1 and CYP19A1 Genes Mutation with the Corresponding Odds Ratio (OR), 95% Confidence Interval (CI), and Significant Association (χ2, p-value) in Cases and Control Groups

		Cases (n=100)	Controls (n=50)	OR (95%CI)	χ^2	p-value
CYP17A1	T/T	68 (68%)	35 (70%)	-	0.072	0.964
Genotypic Frequency	T/C	26 (26%)	12 (24%)			
	C/C	6 (6%)	3 (6%)			
Allelic Frequency	T	162 (81%)	82 (82%)	1.068 (0.574-1.987)	0.043	0.834
	C	38 (19%)	18 (18%)			
CYP19A1	G/G	67 (67%)	43 (86%)	-	0.773	0.208
Genotypic Frequency	G/A	33 (33%)	7 (14%)			
	A/A	0 (6%)	0 (0%)			
Allelic Frequency	G	167 (83.5%)	93 (93%)	2.625 (1.117-6.167)	0.507	0.225
	A	33 (16.5%)	7 (7%)			

OR (95% CI): Presents the odds ratio (OR) and its corresponding 95% confidence interval (CI), providing information on the strength of the association between the genotype/allele and PCOS. χ2: Represents a statistical measure used to assess the significance of differences in allele frequencies between cases and controls. p-value: evaluating the statistical significance of the observed differences in allele frequencies.

Discussion

Polycystic ovarian syndrome is a multifactorial endocrine condition that is causing infertility and anovulation in females throughout the globe [22]. It also increases the risk of developing psychological problems such as depression, due to hyperandrogenism, which causes hirsutism, male body configuration, and obesity [23,24]. Both genetic and environmental factors are involved due to their multifactorial nature

[25]. The present study was conducted to check the effect of different environmental factors and genetic variations on the development of PCOS.

The results of the present study showed that females in the age group of 21-30 years were remarkably more prone to PCOS. Our results are contradictive to the study performed by Talbot et al. (2000), [26] suggesting PCOS was more prevalent among older women as compared to young ones. The variation may be attributed to different ethnic backgrounds and environmental conditions. In contrast to our findings, Guo et al (2007), also reported a significant association between CYP19A1 and age at menarche among Caucasian women [27].

Visceral fat is an important contributor to PCOS. The intermediary role of ectopic fat as a risk factor in PCOS is not completely known. A strong association of PCOS with obesity was observed in the present work. Similar results were reported by Joham et al, (2015), study, showing that in Australia, 70% of the women with PCOS have abdominal obesity [28]. Shan et al. (2015) also reported similar findings showing PCOS association with obesity, insulin resistance, and hyperandrogenism. So obesity has an important role in the development of PCOS [29].

About 5 to 10 percent of women having PCOS also suffer from hirsutism, a cutaneous manifestation that results in anxiety associated with elevated androgens, polycystic ovaries, along with acne [30]. The present study showed 79% cases of hirsutism and 78% cases of acne with PCOS indicating a strong link between these two factors with PCOS.

In the present work, BMI shows a positive association with PCOS ($p<0.05$). Similar results were reported by a study on Indian women, with a significant ($p<0.0001$) association of BMI with PCOS [31]. Zhou, Fang et al., (2017), showed there is a significant association of BMI with PCOS subjects ($p = 0.001$), [32]. The same results were found by Deepika et al. (2013), in South Indian women ($p = 0.0001$), [33]. In contrast to our results Haider et al. (2014), and Shi, Zhao, et al. (2012) reported no significant association of BMI with the disease under study ($p = 0.575$, $p = 0.831$), respectively [34,35].

Polycystic ovarian syndrome (PCOS) is a complex hormonal disorder that affects many women worldwide. While its exact cause remains elusive, researchers have explored the potential role of genetic factors, particularly the CYP17A1 gene, which is involved in steroid hormone synthesis. One specific single nucleotide polymorphism of interest is rs743572, specifically the c-34T/C polymorphism. Several studies have investigated the association between the CYP17A1 SNP and PCOS but have yielded inconsistent results. In the current study, researchers examined the genotypic and allelic frequencies of the rs743572 in both PCOS cases and controls. They found no significant differences between the two groups, suggesting that this particular mutation may not be a direct contributing factor for PCOS [36]. Similarly, other studies in different populations, including Russian [36], Korean [37], and Polish [17,38], have also found no significant link between the CYP17A1 mutation and PCOS. However, it's essential to note that a few studies conducted in India and Iran, reported a significant association between the CYP17A1 mutation and PCOS. These discrepancies highlight the complexity of the condition and the influence of genetic variations in different ethnic groups.

According to our findings of CYP17A1, there is a non-significant link of the disease with this mutation. Our results are similar to the o findings of Park et al. (2008), that found no significant association between CYP17A1 and PCOS [39]. Chua et al. (2012), also reported that genetic variation in the CYP17A1 gene was not a major risk factor for PCOS [40]. Mohammed et al. (2015) study showed a non-significant association of CYP17A1 gene mutation with the development of PCOS in Iraqi women [41]. A similar result was illustrated by Marszalek et al. (2001), in the Polish population, where they genotyped 56 PCOS women and concluded that polymorphism of this gene is not associated with steroid hormone synthesis in PCOS and does not constitute a primary genetic defect [42]. Even though these findings seem to suggest that CYP17A1 is not directly linked with the susceptibility of PCOS, its unusual function may contribute to the series of reactions of the biosynthesis pathways with other genetic abnormalities in PCOS women.

In contrast to the studies suggesting no significant association [43] [44], some research groups, particularly in Pakistans [45] [46], reported a strong correlation between the CYP17A1 mutation and PCOS risk. These studies found a higher prevalence of the mutant allele in PCOS cases compared to controls, indicating a

potential genetic susceptibility in this specific population. However, the reasons for these contrasting results are not entirely clear, and further research is needed to explore the potential genetic heterogeneity of PCOS across different populations. The overall results from various studies, including the current one, leans toward no direct association between CYP17A1 and PCOS so, may not be a primary genetic defect causing PCOS, researchers suggest that it could still play a role in the pathogenesis of this disease when combined with other genetic abnormalities or environmental factors.

Present research work demonstrated that there was no significant association of CYP19A1 (rs2414096) polymorphism with PCOS, similar to Reddy et al.'s (2015), study of South Indian women [47]. A study conducted by Soderlund et al. (2005), examined the distribution of variants in the ovary promoter in 25 PCOS patients and 50 controls and found no evidence of CYP19A1 mutations [48]. According to Nectaria Xita, CYP19A1 polymorphisms are associated with serum testosterone concentrations. The study concluded that CYP19A1 may not be a key genetic factor for determining PCOS, but rather a genetic modifier [49]. In contrast to our results, a study on the Chinese population found a significant association between rs2414096 in CYP19A1 and PCOS susceptibility ($p = 0.001$), [50]. Our findings might suggest that CYP19A1 is not probably the susceptible gene for PCOS and many environmental factors and lifestyles may play an important role in maintaining the hormone level in the female body, so it may not be closely associated with a single factor abnormality.

Conclusion

This study aimed to investigate the genetic association of rs2414096 in the CYP19A1 gene and rs743572 in the CYP17A1 with PCOS in the local female population of District Bahawalpur, Pakistan. There was no significant difference in the genotypic distribution of CYP17A1 alleles and CYP19A1 alleles between patients and controls. This suggested that these polymorphisms are not significantly associated with PCOS in the District Bahawalpur population, but it also concluded that obesity, hirsutism, dysmenorrhea, and acne along with lifestyle and diet could have a strong link with this syndrome as there were remarkable differences in clinical and hyperandrogenic parameters of cases and healthy individuals. PCOS is a heterogeneous condition with multiple genetic and environmental factors, making it challenging to identify a single causative gene or variant.

Further studies should continue to explore the genetic basis of PCOS by investigating multiple genes and variations simultaneously. Collaborative efforts involving large and diverse cohorts from various ethnic backgrounds may provide more comprehensive insights into the genetic underpinnings of this complex syndrome. Understanding the genetic factors contributing to PCOS can pave the way for more personalized and effective treatments in the future.

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Authors' Contribution

Tasleem Kausar[1]* Conceived and designed the experiments, Nadia Noureen[1] critically analyzed the data, Shumaila javed[1] performed lab work, Rubina Shakil[1] enrolled the samples, Fareeha Shahid[1] performed lab work, Sana Abdul Sattar[1] performed statistical analysis, Mahnoor Samra[1] Wrote the paper, Umme Abiha[1] Wrote the paper, Sobia Sadia[2] Draw the graphs and images, Nabeela Tariq[3], Collect the tables data, Amjad Islam[4], critically analyze the data, Israr Maqbool[5] analyze and proofread the data.

Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article

REFERENCES

Asunción M, Calvo RM, San Millán JL, Sancho J, Avila S, et al. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. The Journal of Clinical Endocrinology & Metabolism, (2000); 85(7): 2434-2438.

Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. International journal of andrology, (2006); 29(1): 278-285.

Hossain N, Stepanova M, Afendy A, Nader F, Younossi Y, et al. Non-alcoholic steatohepatitis (NASH) in patients with polycystic ovarian syndrome (PCOS). (2011); 46(4): 479-484.

Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz RJNre. Polycystic ovary syndrome: etiology, pathogenesis, and diagnosis. (2011); 7(4): 219-231.

Homburg RJBP, Endocrinology RC, Metabolism. Pregnancy complications in PCOS. (2006); 20(2): 281-292.

Cocksedge KA, Li T-C, Saravelos SH, Metwally MJRbo. A reappraisal of the role of polycystic ovary syndrome in recurrent miscarriage. (2008); 17(1): 151-160.

Chen J, Wang Q, Cabrera PE, Zhong Z, Sun W, et al. Molecular genetic analysis of Pakistani families with autosomal recessive congenital cataracts by homozygosity screening. Investigative ophthalmology & visual science, (2017); 58(4): 2207-2217.

Li Y, Chen C, Ma Y, Xiao J, Luo G, et al. Multi-system reproductive metabolic disorder: significance for the pathogenesis and therapy of polycystic ovary syndrome (PCOS). Life Sciences, (2019); 228167-175.

Ashraf S, Rasool SUA, Nabi M, Ganie MA, Masoodi SR, et al. Impact of rs2414096 polymorphism of CYP19 gene on the susceptibility of polycystic ovary syndrome and hyperandrogenism in Kashmiri women. Scientific Reports, (2021); 11(1): 1-10.

Kadioglu E, Altun B, İpek Ç, Döğer E, Bideci A, et al. The role of DENND1A and CYP19A1 gene variants in individual susceptibility to obesity in Turkish population—a preliminary study. Molecular biology reports, (2018); 45(6): 2193-2199.

Nelson VL, Legro RS, Strauss III JF, McAllister JM. Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Molecular endocrinology, (1999); 13(6): 946-957.

Takayama K, Suzuki T, Bulun SE, Sasano H, Yilmaz B, et al. Organization of the human aromatase p450 (CYP19) gene; 2004. Copyright© 2004 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New …. pp. 5-9.

Dadachanji R, Shaikh N, Mukherjee S. Genetic variants associated with hyperandrogenemia in PCOS pathophysiology. Genetics research international, (2018); 2018.

de Medeiros SF, Barbosa JS, Yamamoto MMW. Comparison of steroidogenic pathways among normoandrogenic and hyperandrogenic polycystic ovary syndrome patients and normal cycling women. Journal of Obstetrics and Gynaecology Research, (2015); 41(2): 254-263.

Yu Y-Y, Sun C-X, Liu Y-K, Li Y, Wang L, et al. Promoter methylation of CYP19A1 gene in Chinese polycystic ovary syndrome patients. Gynecologic and obstetric investigation, (2013); 76(4): 209-213.

Fan Y-S, Sasi R, Lee C, Winter J, Waterman M, et al. Localization of the human CYP17 gene (cytochrome P45017α) to 10q24. 3 by fluorescence in situ hybridization and simultaneous chromosome banding. (1992); 14(4): 1110-1111.

Li L, Gu Z-P, Bo Q-M, Wang D, Yang X-S, et al. Association of CYP17A1 gene-34T/C polymorphism with polycystic ovary syndrome in Han Chinese population. (2015); 31(1): 40-43.

Diamanti-Kandarakis E, Piperi C, Argyrakopoulou G, Spina J, Papanastasiou L, et al. Polycystic ovary syndrome: the influence of environmental and genetic factors. (2006); 5(1): 17.

Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, et al. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. (2016); 106(1): 6-15.

Amiri M, Ramezani Tehrani F, Nahidi F, Bidhendi Yarandi R, Behboudi- Gandevani S, et al. Association between biochemical hyperandrogenism parameters and Ferriman- Gallwey score in patients with polycystic ovary syndrome: a systematic review and meta- regression analysis. (2017); 87(3): 217-230.

Ye S, Dhillon S, Ke X, Collins AR, Day IN. An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic acids research, (2001); 29(17): e88-e88.

Araújo BS, Baracat MCP, dos Santos Simões R, de Oliveira Nuñes C, Maciel GAR, et al. Kisspeptin Influence on Polycystic Ovary Syndrome—A Mini Review. Reproductive Sciences, (2020); 27(2): 455-460.

Almeshari WK, Alsubaie AK, Alanazi RI, Almalki YA, Masud N, et al. Depressive and anxiety symptom assessment in adults with polycystic ovarian syndrome. Depression research and treatment, (2021); 2021.

Ramezani Tehrani F, Behboudi-Gandevani S, Bidhendi Yarandi R, Saei Ghare Naz M, Carmina E. Prevalence of acne vulgaris among women with polycystic ovary syndrome: a systemic review and meta-analysis. Gynecological Endocrinology, (2021); 37(5): 392-405.

Chen X, Koivuaho E, Piltonen TT, Gissler M, Lavebratt C. Reply: Association of maternal polycystic ovary syndrome or anovulatory infertility with obesity and diabetes in offspring: a population-based cohort study. Human Reproduction, (2022); 37(1): 193-194.

Talbott EO, Guzick DS, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, et al. Evidence for an association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. Arteriosclerosis, thrombosis, and vascular biology, (2000); 20(11): 2414-2421.

Chen M-J, Yang W-S, Yang J-H, Chen C-L, Ho H-N, et al. Relationship between androgen levels and blood pressure in young women with polycystic ovary syndrome. (2007); 49(6): 1442-1447.

Joham AE, Teede HJ, Ranasinha S, Zoungas S, Boyle J. Prevalence of infertility and use of fertility treatment in women with polycystic ovary syndrome: data from a large community-based cohort study. Journal of women's health, (2015); 24(4): 299-307.

Shan B, Cai J-h, Yang S-Y, Li Z-R. Risk factors of polycystic ovarian syndrome among Li People. Asian Pacific journal of tropical medicine, (2015); 8(7): 590-593.

Lowenstein EJ. Diagnosis and management of the dermatologic manifestations of polycystic ovary syndrome. Dermatologic therapy, (2006); 19(4): 210-223.

Thathapudi S, Kodati V, Erukkambattu J, Katragadda A, Addepally U, et al. Anthropometric and biochemical characteristics of polycystic ovarian syndrome in South Indian women using AES-2006 criteria. International journal of endocrinology and metabolism, (2014); 12(1).

Zhou W, Fang F, Zhu W, Chen Z-J, Du Y, et al. Bisphenol A and ovarian reserve among infertile women with polycystic ovarian syndrome. International journal of environmental research and public health, (2017); 14(1): 18.

Deepika M, Reddy KR, Yashwanth A, Rani VU, Latha KP, et al. TNF-α haplotype association with polycystic ovary syndrome–a South Indian study. Journal of assisted reproduction and genetics, (2013); 30(11): 1493-1503.

Haider S, Mannan N, Khan A, Qureshi MA. Influence of anthropometric measurements on abnormal gonadotropin secretion in women with polycystic ovary syndrome. J Coll Physicians Surg Pak, (2014); 24(7): 463-466.

Shi Y, Zhao H, Shi Y, Cao Y, Yang D, et al. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. Nature Genetics, (2012); 44(9): 1020-1025.

Beglova AY, Elgina S, Gordeeva LJOG. Polymorphism of the CYP11A1, CYP17A1, and CYP19A1 genes in reproductive-aged women with polycystic ovary syndrome. (2019); 12148-153.

Park J-M, Lee E-J, Ramakrishna S, Cha D-H, Baek K-HJIjomm. Association study for single nucleotide polymorphisms in the CYP17A1 gene and polycystic ovary syndrome. (2008); 22(2): 249-254.

Dasgupta A, Banerjee U, Roy P, Khan A, Ghosh M, et al. Assessment of CYP 17 gene polymorphism in subjects with Polycystic Ovarian Syndrome and Central Obesity in an Indian Subpopulation. (2014); 14(1): 33-41.

Park J-M, Lee E-J, Ramakrishna S, Cha D-H, Baek K-H. Association study for single nucleotide polymorphisms in the CYP17A1 gene and polycystic ovary syndrome. International journal of molecular medicine, (2008); 22(2): 249-254.

Chua AK, Azziz R, Goodarzi MO. Association study of CYP17 and HSD11B1 in polycystic ovary syndrome utilizing comprehensive gene coverage. MHR: Basic science of reproductive medicine, (2012); 18(6): 320-324.

Mohammed MB, AL-Awadi SJ, Omran MA. Association between polycystic ovary syndrome and genetic polymorphisms of CYP 17 gene in Iraqi women. Iraqi journal of biotechnology, (2015); 14(2).

Marszalek B, Laciński M, Babych N, Capla E, Biernacka-Lukanty J, et al. Investigations on the genetic polymorphism in the region of CYP17 gene encoding 5′-UTR in patients with polycystic ovarian syndrome. Gynecological endocrinology, (2001); 15(2): 123-128.

Kaur R, Kaur T, Kaur AJJoar, genetics. Genetic association study from North India to analyze the association of CYP19A1 and CYP17A1 with polycystic ovary syndrome. (2018); 35(6): 1123-1129.

Rahimi Z, Mohammadi EJIJoRB. The CYP17 MSP AI (T-34C) and CYP19A1 (Trp39Arg) variants in polycystic ovary syndrome: A case-control study. (2019); 17(3): 201.

Munawar Lone N, Babar S, Sultan S, Malik S, Nazeer K, et al. Association of the CYP17 and CYP19 gene polymorphisms in women with polycystic ovary syndrome from Punjab, Pakistan. (2021); 37(5): 456-461.

Aziz N, Doggar S, Shoaib A, Aslam MS, Altaf MOJP. Single nucleotide polymorphisms (CYP11 ALPHA AND CYP17) and serum sex hormone binding globulin levels in normal and polycystic ovary syndrome. (2021); 71(4): 1484-1488.

Joseph N, Reddy AG, Joy D, Patel V, Santhosh P, et al. Study on the proportion and determinants of polycystic ovarian syndrome among health sciences students in South India. Journal of natural science, biology, and medicine, (2016); 7(2): 166.

Söderlund D, Canto P, Carranza-Lira S, Mendez J. No evidence of mutations in the P450 aromatase gene in patients with polycystic ovary syndrome. Human Reproduction, (2005); 20(4): 965-969.

Xita N, Lazaros L, Georgiou I, Tsatsoulis A. CYP19 gene: a genetic modifier of polycystic ovary syndrome phenotype. Fertility and sterility, (2010); 94(1): 250-254.

Jin J-L, Sun J, Ge H-J, Cao Y-X, Wu X-K, et al. Association between CYP19 gene SNP rs2414096 polymorphism and polycystic ovary syndrome in Chinese women. BMC medical genetics, (2009); 10(1): 1-5.