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# ASSOCIATION OF REPRODUCTIVE HORMONES AND GENE POLYMORPHISMS WITH POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS – A HOSPITAL-BASED PILOT STUDY

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**Abstract:**

Polycystic ovary syndrome (PCOS)impacts approximately 18% of women globally, significantly affecting their reproductive health. This condition is marked by chronic anovulation, elevated levels of androgens, and the presence of multiple cysts on the ovaries. The present study aims to investigate reproductive hormonal levels and gene polymorphisms in PCOS patients. The study population consisted of 20 PCOS patients and 20 healthy controls. Hormonal (Luteinising hormone (LH), follicle-stimulating hormone (FSH), testosterone, estrogen, and progesterone) levels were analysed using sandwich and competitive ELISA techniques, while genotypic analysis ofCYP17A1, CYP19A1, ESR1, and ESR2 genes was conducted using polymerase chain reaction and Sanger sequencing. Significant differences in BMI were observed between subjects and controls. Luteinising hormone (LH), follicle-stimulating hormone (FSH), LH: FSH ratio, testosterone and estrogen levels were significantly elevated in PCOS patients, whereas progesterone levels were significantly decreased compared to healthy controls. However, no significant differences were observed in CYP17A1, CYP19A1, ESR1, and ESR2 gene polymorphisms between PCOS patients and controls. In conclusion, our study suggests that altered levels of LH, FSH, testosterone, estrogen, and progesterone were significantly associated with the development of PCOS, while gene polymorphisms did not show any association with the condition.

**Keywords:** Polycystic ovary syndrome (PCOS), Luteinising hormone (LH), follicle-stimulating hormone (FSH), testosterone, estrogen, and progesterone levels CYP17A1, CYP19A1, ESR1, and ESR2 gene polymorphisms

**Introduction:**

Polycystic ovary syndrome (PCOS), also known as hyperandrogenic anovulation or Stein-Leventhal syndrome, is a hormonal disorder characterized by various symptoms, including irregular menstrual cycles (amenorrhea), the presence of multiple small cysts (polycystic ovaries), infertility, excessive facial hair growth (hirsutism), hyperthecosis, and increased production of androgens (Mariya Kamil et al., 2020). PCOS affects 6-18% of women (Teede et al., 2013), and is a leading cause of ovary dysfunction. According to the Rotterdam Consensus 2003, a diagnosis of polycystic ovary syndrome (PCOS) is typically made when at least two out of the following three features are present 1) Oligo- anovulation 2) Hyperandrogenism and 3) Polycystic ovarian morphology (Azziz et al., 2006).

The diagnosis of polycystic ovarian morphology (PCOM) involves the presence of 12 or more antral follicles with diameters ranging from 2 to 9 mm, along with increased ovarian volume (Jonard et al., 2003). PCOS is a multifaceted endocrine disorder that not only impacts women's reproductive health but also predisposes individuals to various chronic disorders, including obesity, insulin resistance, sleep apnea, cardiovascular disorders (CVD), and other metabolic disorders (Cobin et al., 2013).

Disrupted menstrual cycles and hormonal imbalances are common symptoms observed in patients with PCOS. Abnormal pulsation of gonadotropin hormone-releasing hormone (GnRH) increases LH to FSH levels; inadequate FSH causes failed ovulation and therefore the formation of antral follicles in ovaries. Atypical estrogen levels are responsible for irregular feedback mechanisms that cause an increase in LH levels (Saadia 2020). Higher levels of LH induce the production of androgens from theca cells, which leads to hyperandrogenaemia in PCOS patients (Cadagan et al., 2016).

Genes involved in the steroid synthesis pathway play a crucial role in the development of PCOS, especially cytochrome P450 (CYP) and ESR genes. Among CYP genes, CYP17A1, CYP19A1, ESR1, and ESR2 were included in our present study. CYP17A1 enzyme performs both 17α-hydroxylase and 17,20-lyase activities, which are crucial for the biosynthesis of androgens. This enzyme catalyses the conversion of progesterone and pregnenolone into androstenedione and dehydroepiandrosterone, respectively, in a two-step process. Studies have reported that increased 17α-hydroxylase activity has been associated with hyperandrogenism, marked by elevated levels of androgens, such as testosterone, in the body. This dysregulation in gene expression may contribute to the hormonal imbalances observed in PCOS, including increased androgen production.

Reduced aromatase activity is responsible for increased androgen levels as it leads to decreased conversion of androgens into estrogens. The aromatase enzyme is encoded by the CYP19A1 gene, which is located on chromosome 15. Mutation in the CYP19A1 may lead to the development of polycystic ovarian syndrome (PCOS) (Nasira Munawar Lone et al., 2020).

The ESR1 gene is located on chromosome 6, while the ESR2 gene is on chromosome 14. ESR1 codes for estrogen receptor alpha and ESR2 codes for estrogen receptor beta. receptors regulate estrogen function during ovulation and follicle development. Both receptors play a crucial role in maintaining hypothalamus-pituitary-ovarian axis function (Necteria et al., 2012).

**Materials and Methods:**

**Study population:** This study includes 20 PCOS patients and 20 healthy controls. The study was carried out at the Bhagwan Mahavir Medical Research Centre (BMMRC). The participants were explained about the purpose of the present study, and consent forms were obtained from them. The study was approved by the Ethics Committee of BMMRC. Blood samples were collected from the participants who had given their consent to participate in the study. Subjects and controls are asked to complete questionnaires regarding information about their age, sex, menstrual cycle, habits, and health condition. This information was collected from the PCOS patients and control subjects using a standard questionnaire prepared for this study.

**Inclusion criteria:**

Women aged between 16 and 35 were experiencing struggles related to PCOS, obesity, irregular menstrual cycles, and hyperandrogenism (hirsutism).

**Exclusion criteria:**

Married women and those above 35 years of age and PCOS patients currently undergoing medication for PCOS treatment were excluded. Women with other androgenic disorders such as Cushing's syndrome or pre-ovarian failure were also excluded.

**Sample collection:** 5ml of blood sample was collected from both patients and controls into EDTA coated vacutainer. Subsequently, 3ml of blood from each sample was collected in a fresh plain vacutainer and allowed to separate to obtain serum. The serum was then transferred into fresh 2 ml centrifuge tubes and stored at -80 ºC for further analysis.

**DNA extraction:**

The salting out method was used to isolate genomic DNA from whole blood. A biophotometer was used to measure the concentration and purity of the isolated genomic DNA. Gel electrophoresis was employed for quantification.

**Polymerase chain reaction:**

In this study, a gradient PCR was used to amplify specific regions of four selected genes: CYP17A1, CYP19A1, ESR1, and ESR2. In this study, primers were designed for the amplification of specific regions within genes relevant to the investigation. For the CYP17A1 gene, the forward primer 5'-GTCAAGGTGAAGATCAGGGTAG-3' and the reverse primer 5'-TGGTCTGAAGACCTGAACAATC-3' were utilized, resulting in a product size of 473 base pairs (bp). These primers targeted the 5' untranslated region (5'UTR) and Exon 1 of the CYP17A1 gene. Similarly, for the CYP19A1 gene, the forward primer 5'-AGAGCAACCTTCTTAGGCTCAC-3' and the reverse primer 5'-AAAGTTACCTGAGAGGCCAAG-3' were employed, generating a product of 282 bp. This amplification targeted Exon 7 of the CYP19A1 gene.

In the case of the ESR1 gene, amplification was carried out using the forward primer 5'-TCTCCCAGAGAGTGCATGTT-3' and the reverse primer 5'-TCAGTCGCTTTGGCTCTTAGG-3', resulting in a product size of 324 bp. The primer was designed to target Exon 2 of the ESR1 gene. Lastly, for the ESR2 gene, the forward primer 5'-AACAGCTGAGCACACGACTT-3' and the reverse primer 5'-CCGTGGAGCACATAATCCCA-3' were utilized, producing a product of 308 bp. This primer was designed to target the 3' untranslated region (3'UTR) and Exon 8 of the ESR2 gene. After amplification, PCR products were quantified using gel electrophoresis and a biophotometer. Further PCR products of genes were sequenced by the Sanger sequencing method and single nucleotide polymorphisms (SNPs) were studied using bioinformatic tools.

**Enzyme-linked immunosorbent assay (ELISA):**

Quantitative and qualitative analysis of hormones, including LH, FSH, Testosterone, Estrogen, and Progesterone, was conducted using Enzyme-Linked Immunosorbent Assay (ELISA). The study focused on assessing the fluctuations of the above hormones in patients with PCOS. Sandwich Elisa was used for the quantitative analysis of LH and FSH, while Competitive Elisa was employed for estrogen, progesterone and testosterone analysis (Fine Test ELISA kits, USA/China)

**Statistical analysis:**

The means of the two independent samples for each hormone were compared using a t-test. This statistical method is appropriate for comparing means between two groups when the data is assumed to be normally distributed.

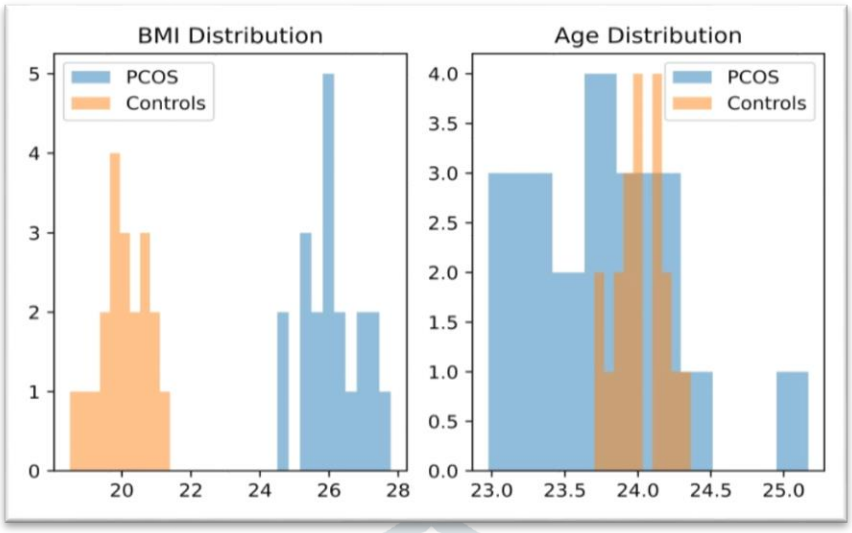
**Results:**

In the present study, we investigated levels of LH, FSH, estrogen, progesterone, and testosterone hormones as well as polymorphisms of CYP17A1(C-T), CYP19A1(A-C), ESR1(A-T), and ESR2(C-G) genes along with various demographic and clinical parameters.

**Table 1: Age and BMI distribution in PCOS patients and controls.**

| Parameter | PCOS patients (Mean±SD) | Controls (Mean±SD) | P-Value |
|---|---|---|---|
| Age | 24±0.6 | 24.1±0.23 | 0.05* |
| BMI | 25.5±1.02 | 20.03±0.6 | 0.000** |

**Fig 1: The histogram depicts the distribution of BMI and age for both PCOS patients and healthy controls.**



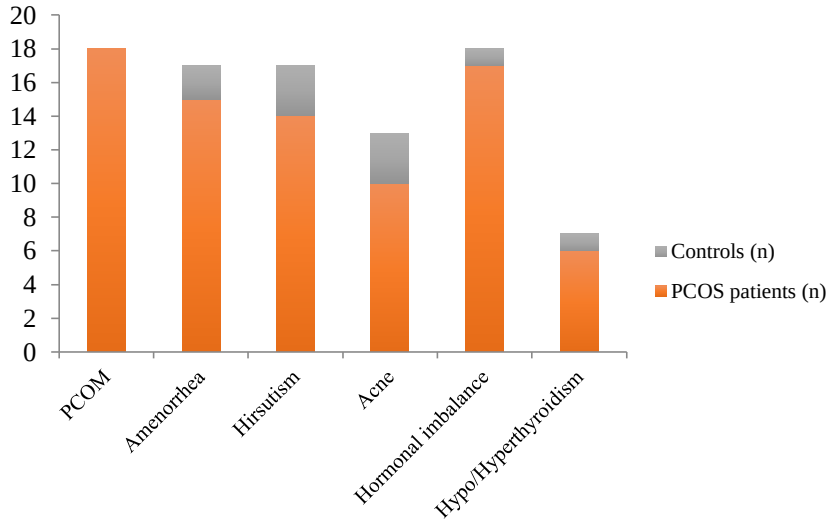

The mean age observed in the PCOS patients was 24±0.6, in the controls it was 24.1± 0.23(Table 1). Participants were divided into different age groups. All participants were within the reproductive age range. Regarding BMI, the mean value among subjects was 25.5±1.02, whereas among controls it was20.03±0.6(Table 1). A higher proportion of PCOS patients were categorized as overweight or obese when compared to controls. The p-value for age is close to 0.05, suggesting that the difference in age between the two groups is not significant. Conversely, the p-value for BMI is notably low, indicating a statistically significant difference in BMI between PCOS patients and the control group (Table 1).

Figure 1 depicts the distribution of BMI and age for both PCOS patients and healthy controls. For age, the distributions are quite similar, which corresponds to the marginal p-value, suggesting only a slight difference that may lack clinical significance. For BMI, the PCOS group exhibits a higher mean and a wider spread compared to the control group, consistent with the significant p-value indicating a genuine difference.

**Table 2: Clinical representation in PCOS patients and controls**

| Parameter | PCOM | Amenorrhea | Hirsutism | acne | Hormonal imbalance | Hypo/Hyperthyroidism |
|---|---|---|---|---|---|---|
| **PCOS patients(n) (%)** | 18(90) | 15(75) | 14(70) | 10(50) | 17(85) | 6(30) |
| **Controls (n) (%)** | 0(0) | 2(10) | 3(15) | 3(15) | 1(5) | 1(5) |

**Figure 2: Clinical representation in PCOS patients and controls**




Polycystic ovary syndrome is characterized by polycystic ovary morphology, Hirsutism, and irregular menstrual cycles. Elevated androgen levels lead to acne, male-pattern facial hair and hormonal imbalance. Certain clinical features serve as diagnostic criteria for PCOS. As depicted in Table 2 and Figure 2 majority of PCOS patients exhibit PCOM, Amenorrhea, Hirsutism, Acne, Hormonal imbalances and hypo/hyperthyroidism. PCOM is completely absent in healthy controls, and other clinical features are seen only in a few cases of controls.

**Table 3: Hormonal levels in PCOS patients and controls.**

| Parameter | PCOS Patients (Mean±SD) | Controls (Mean±SD) | p-value |
|---|---|---|---|
| **Estrogen (pg./mL)** | 105.72±4.32 | 59.15±2.6 | 0.000*** |
| **Progesterone(ng/ mL)** | 1.29±0.3 | 5.35±0.3 | 0.000*** |
| **Testosterone(ng/ mL)** | 14.27±3.31 | 0.242±0.08 | 0.000*** |
| **LH (mIU/ mL)** | 18.832±1.78 | 6.01±0.5 | 0.000*** |
| **FSH (mIU/ mL)** | 12.55±1.35 | 5.79±0.55 | 0.000*** |
| **LH: FSH** | 1.50±1.3 | 1.03±0.9 | 0.002*** |

**Fig3: The histogram represents the distribution of various hormonal levels in both PCOS patients and healthy controls.**

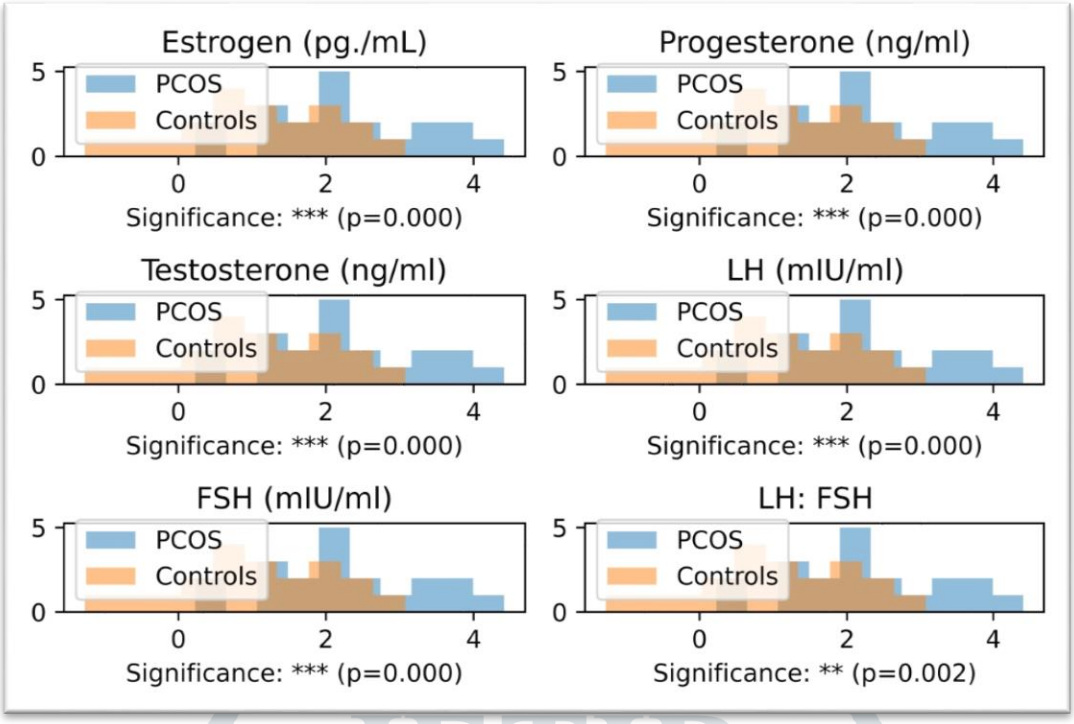



Table 3 shows mean hormonal levels and standard deviations along with p-value for both PCOS patients and controls. The mean level of Estrogen (pg/mL) observed in PCOS patients was 105.72±4.32, while in controls it was 59.15±2.6. A significant increase ($p<0.05^{**}$) in estrogen levels was observed in subjects compared to controls (Fig 3, Table 3). The mean level of progesterone (ng/ml) was 1.29±0.3 in subjects and 5.35±0.3 in controls. There was a significant decrease ($p<0.05^{**}$) in progesterone levels observed in subjects compared to controls (Table 3 and Figure 3).

The mean value of Testosterone(ng/ml) level was 14.27±3.31 in subjects and 0.242±0.08 in controls. Levels of Testosterone were significantly ($p<.05^{**}$) higher in subjects than in controls (Table 3 and Figure 2). Similarly, the mean value of LH (mIU/ml) level was 18.832±1.78 in subjects and 6.01±0.5 in controls. LH significantly ($p<.05^{**}$) increased in subjects compared to controls (Fig 2, Table 3). The mean value of FSH (mIU/ml) level was 12.55±1.35 in subjects and 5.79±0.55 in controls. Subjects exhibited significantly elevated FSH levels compared to controls. Additionally, LH: FSH ratio is 1.50±1.3 in subjects and 1.03±0.9 in controls. The ratio significantly increased in subjects compared to healthy controls (Table 3 and Figure 3).

**Table 4: Gene polymorphisms in PCOS patients and controls.**

| Parameter | PCOS patients n=20(%) | Controls n=20(%) | $\chi^2$ | P value |
|---|---|---|---|---|
| **CYP17A1(C-T** | | | | |
| CC | 5(25) | 8(40) | 1 | |
| CT | 10(50) | 10(50) | 0.09 | $0.722^{ns}$ |
| TT | 5(25) | 2(10) | 0.879 | $0.3484^{ns}$ |
| C Allele | 20(50) | 26(65) | | |
| T Allele | 20(50) | 14(35) | | |
| **CYP19A1(A-C** | | | | |
| AA | 9(45) | 14(70) | 1 | |
| AC | 9(45) | 4(20) | 1.926 | $0.1652^{ns}$ |
| CC | 2(10) | 2(10) | 0.167 | $0.683^{ns}$ |
| A Allele | 27(67.5) | 32(80) | 1 | |
| C Allele | 13(32.5) | 8(20) | | |
| **ESR1(A-T)** | | | | |
| AA | 4(20) | 6(30) | 1 | |
| AT | 9(45) | 11(55) | 0.068 | $0.7945^{ns}$ |
| TT | 7(35) | 3(15) | 0.808 | $0.3687^{ns}$ |
| A Allele | 17(42.5) | 23(57.5) | 1 | |
| T Allele | 23(57.5) | 17(42.5) | | |

| | | | | |
|---|---|---|---|---|
| **ESR2(C-G)** | | | | |
| CC | 7(35) | 9(45) | 1 | |
| CG | 10(50) | 10(50) | 0.001 | 0.9702 ns |
| GG | 3(15) | 1(5) | 0.313 | 0.5762 ns |
| C Allele | 24(60) | 28(70) | 1 | |
| G Allele | 16(40) | 12(30) | | |

CYP17A1, CYP19A1, ESR1, and ESR2 gene polymorphisms were studied and the details of genotypes and allele frequencies are presented in Table 4. The amplified DNA fragments were sequenced using Sanger sequencing. Polymorphisms were observed in four different genes CYP17A1, CYP19A1, ESR1, and ESR2. The enzyme encoded by the CYP17A1 gene possesses dual functions, 17-hydroxylase and 17,20-lyase, and it plays a crucial role in steroid hormone synthesis. The Observed CYP17A1 genotypes included CC (homozygous, wild), CT (heterozygous) and TT (Mutant, homozygous). In the subjects, the distribution of genotypes was 25% CC, 50% CT, and 25% TT, while in the controls it was 40% CC, 50% CT, and 10%TT. The aromatase enzyme is encoded by the CYP19A1 gene, which is responsible for converting androgen into estrogen hormones. The observed genotypes of CYP19A1 were (Wild type, homozygous), AC (heterozygous) and CC (mutant). In the subjects, the distribution of genotypes was 45%AA,45%AC, and 10%CC, while in the controls, it was 70%AA,20%AC and 10%CC. There was no significant difference in CYP19A1 polymorphism between PCOS cases and controls.

In the present study, polymorphisms in the ESR1 and ESR2 genes were investigated. For ESR1, the observed genotypes are AA (homozygous, wild type), AT (heterozygous), and TT (homozygous mutant). In both subjects and controls, the distribution of genotypes was 20%AA, 45%AT, and 35%TT, and 30%AA, 55%AT, and 15%TT respectively. For ESR2, the observed genotypes are CC (homozygous, wild type), CG (heterozygous), and GG (homozygous mutant). In subjects, the distribution of genotypes was 35%CC, 50%CG, and 15%GG, as against 45%AA, 50%AC, and 5%CC in the controls. The polymorphisms of CYP17A1, CYP19A1, ESR1, and ESR2 genes showed no significant difference between PCOS patients and controls.

**Discussion:**

In the present study, we observed high BMI in the patient group compared to controls. Sara Rosenberg (2019) also observed increased BMI in PCOS patients and concluded girls who are obese in childhood are more prone to have oligo- anovulation in future. Soydinc et al., (2013) also concluded that obese PCOS patients have increased epicardial thickness compared to lean controls. However, Bulent O. Yildizet al., (2008) observed that the risk of PCOS is more or less similar in both obese and lean women and concluded that it mainly depends on environmental factors.

In the present study, we found higher levels of LH, FSH and LH: FSH ratio in patients when compared to healthy controls. The abnormal hypothalamus-pituitary-ovarian axis leads to a disturbed pattern of GnRH, which in turn induces increased secretion of LH over FSH (Chaudhary et al. 2021). In a study conducted by Saadia in 2020 in Saudi Arabia, patients with PCOS also reported elevated levels of LH, FSH and LH: FSH ratio when compared to healthy controls. Similarly, Malini et al., (2018) observed increased LH, FSH levels and LH: FSH ratio levels in PCOS patients from Kerala. Contrary to these findings, studies conducted by Christine Corter-Rudelli et al., (2002) and Virginie Desforges-Bullet (2010) in France reported decreased levels of FSH in PCOS subjects compared to controls.

In PCOS patients, testosterone and estrogen levels are notably elevated due to increased LH stimulating theca cells to produce androgens, while FSH facilitates the conversion of androgens into estrogens. The disrupted feedback mechanism of estrogen further sustains higher LH levels (Saadia, 2020). Studies by Elena Khashchenko et al. (2020) in Moscow, Russia, Malini (2017) in Kerala, India, and Elisabeth Lerch Baum et al. (2014) in Austria consistently reported elevated levels of both estrogen and testosterone in PCOS patients. The corpus luteum, a temporary gland formed from the follicle after ovulation, secretes progesterone. However, failed ovulation in PCOS patients leads to decreased progesterone levels. Similarly, studies conducted by Malini et al., (2018) in Kerala and Zelija Velija-Ašimi (2013) in Bosnia reported decreased levels of progesterone. In contrast, Christine A. Eagleman et al., (2000) conducted a study in Virginia and reported that no significant differences were observed in PCOS patients, while Linda Schattmann et al., (2007) reported increased progesterone levels in PCOS patients from Quebec, Canada.

In this study, we investigated polymorphisms in CYP17A1, CYP19A1, ESR1, and ESR2 genes. This study is the first of its kind to find out whether there is an association between CYP17A1, CYP19A1, ESR1, and ESR2 gene polymorphisms and the risk of PCOS in women from Telangana state. Similarly, Nasira Munawar Lone et al (2020) reported polymorphisms in CYP17A1 (rs743572) and CYP19A1 (rs2414096) in Punjab, Pakistani women and concluded that C allele of CYP17A1 and A alleles of CYP19A1 is significantly over transmitted in PCOS patients compared to control subjects and significant difference of TC among PCOS patients could be associated with the risk of PCOS. Sairish Ashraf et al (2021) also examined polymorphisms in CYP19A1 (rs2414096) in Kashmiri women and they concluded that polymorphisms at the CYP19 gene may be associated with increased PCOS risk.

Izabela Uzar et al. (2022) observed ESR1(rs3020314 and 1884051) gene polymorphisms in young women and concluded that ESR gene polymorphisms may be associated with the hormonal disturbances that lead to the development of PCOS. Lasma lidaka et al. (2021) investigated single nucleotide variants in ESR2 (rs4986938), which is a non-coding region and reported the presence of a higher level of T in homozygous minor allele carriers but no statistical significance was shown in the development of PCOS. However, in the present study, we did not observe any significant association of polymorphisms in selected regions of respective genes with PCOS. This variation may be due to the gene region selected by different investigations.

**Conclusion:**

In our present study, we observed a significant decrease in progesterone levels in PCOS subjects compared to healthy controls, while Estrogen, LH, FSH and Testosterone levels were significantly elevated in the PCOS patients. Significant differences were observed in BMI between PCOS patients and healthy controls. However, no significant polymorphism in genes between PCOS Subjects and Healthy controls. Based on these findings, we conclude that altered levels of hormones may lead to Polycystic ovary syndrome (PCOS). Our study included a relatively small sample size of only 20 patients and 20 healthy controls. Thus, further study in a larger sample size is warranted to arrive at a definitive conclusion on the association of hormonal levels, and gene polymorphisms with PCOS development.

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**Conflict of interest:**

None of the authors of this paper had any personal or financial conflicts of interest.

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