2. Associations of HSD17B1 gene expression with its DNA methylation and estradiol level in PCOS Indonesian patients

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OnLine Journal of Biological Sciences

Original Research Paper

Associations of HSD17B1 Gene Expression with its DNA Methylation and Estradiol Level in Polycystic Ovary Syndrome Indonesian Patients

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Abstract: PCOS's origin and mechanism are still unknown. Epigenetics has been linked to PCOS in an increasing number of studies in recent years. The most extensively researched epigenetic alteration is DNA methylation. During organismal development, DNA methylation can control gene expression by altering transcription factor binding. The alterations in DNA methylation are directly associated with follicular development in PCOS. Studies show that increased levels of pregnenolone and estrogen in the follicular fluid may affect follicle formation in PCOS patients; the process is largely associated with the expression of HSD17B1. There is evidence to suggest that these levels may have an impact on follicle development in PCOS patients. The mechanism for this effect is partially linked to HSD17B1 expression, which catalyzes the final step in estrogen biosynthesis, 17β-estradiol (E2). We speculated that defects in DNA methylation increase gene dysregulation, resulting in decreased mRNA expression of HSD17B1, which eventually generates insufficient E2 in PCOS patients. The objective of this study is to investigate DNA methylation, mRNA expression, and E2 level in PCOS patients and healthy women groups; the correlation between DNA methylation and mRNA expression in PCOS patients; and the correlation between mRNA expression and E2 serum level in PCOS patients. We provided informed consent to participants; we studied 60 female patients, 30 PCOS patients and 30 healthy women served as the control group, we used the Methyl-Specific PCR (MSP) method and quantitative PCR (qPCR) for DNA methylation and mRNA expression analyses, respectively; and we examined E2 serum levels and hormonal levels. The methylation of the HSD17B1 gene in PCOS women was 42.64% and a healthy group showed 53.80% ($p = 0.160$). The two groups' differences were not statistically significant. The relative expression value of the HSD17B1 gene was 0.70-fold lower compared with the healthy women ($p = 0.003$) group. Significant variances were between the two groups. The average E2 serum level in the PCOS group is 25.78 pg/mL and in the healthy women group, it is 36.74 pg/mL. Compared to the group of healthy women, the PCOS group had a decreased

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E2 serum level. The correlation of DNA methylation level versus mRNA expression in PCOS patients is not significant. ($p = 0.076$). A significant negative association has been seen between the mRNA. There is a significant negative correlation between the mRNA expression of the HSD17B1 gene and serum E2 levels. ($p = 0.020$). "The more down-regulated mRNA expression of the HSD17B1 gene, the lower serum E2 levels." The integrated analysis in this study was hypomethylated DNA and down-regulated mRNA expression of HSD17B1 genes. The hypomethylated DNA was not involved in down-regulating mRNA expression. Therefore, down-regulated mRNA expression of the HSD17B1 gene in PCOS patients can cause lower E2 levels in PCOS, preventing cell growth and potentially contributing to the cause of PCOS pathogenesis.

Keywords: HSD17B1 Gene, DNA Methylation, mRNA Expression, Estradiol, Polycystic Ovary Syndrome

Introduction

The most prevalent metabolic and endocrine condition affecting women of reproductive age is called Polycystic Ovary Syndrome, (PCOS). However, with the scientific community's growing interest neither the pharmaceutical industry nor international health authorities have made comparable advances in PCOS. 1935 saw the first description of PCOS as a combination of obesity, enlarged cystic ovaries, amenorrhea, persistent anovulation infertility, and hirsutism (Escobar-Morreale, 2018).

Clinical trial registration in PCOS is clearly seen at this time. Although there are similar global prevalences for both PCOS and diabetes mellitus, there were only 28 commercial research on PCOS registered at ClinicalTrials.gov in 2017 compared to 4,632 studies on the latter condition. PCOS is not well understood by patients, doctors, or scientists and there is a widespread misconception about its long-term effects. This could be the reason for the lack of interest in the disease. Some explanations could be that it is too diverse to be adequately named, that its definition is debatable, that its etiology and pathophysiology are unknown, or that it is too general to be defined (Escobar-Morreale, 2018).

The World Health Organization (WHO) (2023) estimates that 8-13% of women who are of reproductive age have PCOS; globally, up to 70% of afflicted women do not receive a diagnosis (Abbott *et al*., 2019; Bellver *et al*., 2018; Carbone *et al*., 2019). In Indonesia, there is a lack of information about the number of PCOS cases. As a result, determining which city has the highest frequency of occurrence is challenging. The information on PCOS cases is based on medical records from hospitals in each province.

However, the researcher's interest in PCOS keeps growing, both genetic and epigenetic. Epigenetic variables have been linked to PCOS in an increasing number of studies in recent years (Dyke *et al*., 2019). The most extensively researched epigenetic alteration is DNA methylation. Liu *et al*. (2022) DNA methylation in PCOS has been considered a fresh biological target for creating effective diagnostic markers for predicting PCOS risk or its progression Smirnov *et al*. (2023); Sarkies (2020). In order to adapt to changes in the environment and in lifestyle, DNA methylation has the ability to alter the way genes are expressed without altering the sequence of DNA (Li *et al*., 2020; Hosseini *et al*., 2019; Concha *et al*., 2017). Gene silencing and activation are linked to hypermethylation and hypomethylation in DNA, respectively (Guéant *et al*., 2020; Rotondo *et al*., 2018). Promoter regions are where methylation patterns are found. Through its impact on transcription factor binding during organismal development, DNA methylation has the ability to influence gene expression (Liu *et al*., 2020).

Studies reveal that high levels of pregnenolone and estrogen in follicular fluid may affect follicle formation in PCOS patients; the process is largely associated with the expression of HSD17B1 (Yu *et al*., 2021). Investigate expression changes of the steroidogenic enzyme PCOS. The results of the PCOS rat model showed that, in comparison to the normal control group, the PCOS group exhibited elevated levels of 3β-HSD and 17β-HSD mRNA and protein expressions. According to these findings, the current rat model of PCOS may include 3β-HSD and 17β-HSD in the control of ovarian hormones (Lin *et al*., 2013). The hydroxysteroid 17-beta dehydrogenase-1 (HSD17B1) gene, chromosome 17: 42,549,214-42,555,213 is home to the hydroxysteroid 17-beta dehydrogenase-1 (HSD17B1) gene, type I marker locus (D17S934). (17)

Due to its ability to selectively reduce the weak estrogen Estrone (E1) to produce the powerful estrogen 17β-Estradiol (E2), the HSD17B1 gene is known as the "estrogenic" 17β-HSD. During reproduction, the majority of E2 in females is generated by ovarian granulosa cells through the aromatization of androstenedione, which is generated in theca follicular cells, to E1. Subsequently, 17β-HSD transforms E1 into E2 (Homer *et al*., 2017; Konings *et al*., 2018). Because of the strong correlation

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between the HSD17β gene and E2, this gene is one of the best candidates to be investigated in PCOS's pathogenesis.

Individuals with PCOS have ovarian defects like impaired folliculogenesis, poor oocyte quality, and anovulation that lead to infertility (Budihastuti *et al*., 2019; Amiri *et al*., 2020; Zhang *et al*., 2019; Sagvekar *et al*., 2019). Granulosa cells were gathered as samples because they help the oocyte and theca cells communicate. Theca cells are controlled by the hormones FSH and LH, which stimulate the production of estrogen and "support" the oocyte (Ai *et al*., 2019). Lack of E2 may indicate insufficient granulosa cell development in this study since granulosa cells release E2 mostly in follicles. Anovulation may result from low E2 levels, which have been proposed as a predictor of follicle development (Huang *et al*., 2018).

Numerous research studies have linked HSD17B1 to PCOS, including the association between SNP-71G in type 5 of the 17βHSD polymorphism and androgen excess in some PCOS patients. The HSD17B6 gene's SNP rs898611 has been linked to PCOS's metabolic phenotype. In Chinese women, ovarian steroidogenesis has been linked to elevated expression of HSD17B6 in theca cells of follicles with PCOS; SNP rs1937845 of HSD17B5 is strongly connected with PCOS (Shaaban *et al*., 2019).

We speculated that defects in DNA methylation increase gene dysregulation, resulting in decreased mRNA expression of HSD17B1, which eventually generates insufficient E2 in PCOS patients. We aim to investigate DNA methylation, mRNA expression, and E2 level in PCOS patients and healthy women groups; the relationship between PCOS patients' DNA methylation and mRNA expression; and the relationship between PCOS patients' mRNA expression and E2 serum level.

The only research by Wang *et al*. (2014) found a correlation between hypomethylated DNA and up-regulated mRNA expression of the HSD17B1 gene in PCOS using fresh ovarian tissue in the Chinese population (Wang *et al*., 2014). The research on the associations of HSD17B1 gene expression with its DNA methylation and estradiol level in PCOS Indonesian patients has never been explored. This is the first study to examine DNA methylation and mRNA expression of the HSD17B1 gene in granulosa cells along with E2 serum levels in Indonesian patients.

Materials and Methods

Ethical Statements

The Helsinki Declaration of 1975 and ethical guidelines were followed in the conduct of the inquiry.

Sample Size Calculation

The Lameshows (1990) calculation formula was used to determine the sample size for this study. The prevalence of PCOS is 45.7% among women within the age of reproduction at Dr. Cipto Mangunkusumo Hospital Indonesia's National Reference Center Hospital a leader in services, education, and research.

The lame show's formula:

$$N = \frac{(Za)^2\, p(1-p)}{d2}$$

$$= \frac{(1.96)^2\, 0.457(1-0.457) = 23.83}{(0.2)^2}$$

Description:
N = Total sample number,
$Z\alpha$ = Error rate (1,96)
p = PCOS ratio (10%)
d = Precision (20%)

The total number of samples used in this calculation was 60 people divided into two subjects; we looked at 30 PCOS patients, 30 healthy women, and 60 female patients overall.

Respondent Criteria

1. Healthy women as the control group:

a. Inclusion criteria
- Women who underwent ovum pick-up as part of the IVF procedure had normal ovarian function and no signs of PCOS symptoms
- Male factors cause infertility
- Women with non-patent tubes or tube diseases
- Women who agree to participate in the study sign a consent form after being informed about it

b. Exclusion criteria
- Women with endometritis
- Endometriosis-affected females
- Endometrial cancer sufferers
- Women who have ovarian cancer

2. PCOS group:

a. Inclusion criteria
- Women who had PCOS based on Rotterdam criteria identified through laboratory and ultrasound testing and were undergoing IVF
- Women who agree to participate in the study sign a consent form after being informed about it

b. Exclusion criteria
- Cushing's syndrome in females
- Women with endometritis
- Endometriosis-affected females
- Endometrial cancer sufferers
- Women who have ovarian cancer

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DNA Methylation Level of the HSD17B1 *Gene*

Electrophoresis MSP results (Fig. 1) are representative of 5 samples from the PCOS group and 5 samples from the healthy women group. The methylated HSD17B1 gene in granulosa cells from the 30 PCOS patients was 42.64% and from 30 healthy women (control) it was 53.80% (Fig. 2). The Mann-Whitney test statistical analysis of nonparametric data revealed no significant difference between the two groups (p = 0.160) at p<0.05.

DNA methylation is attracting a lot of attention in studies, which raises a lot of issues like, "How does a transcription pattern become stable?" If specific genes are not active, how can a lens cell remain a lens cell? How do cells go through several cycles of mitosis while remaining differentiated? It's called DNA methylation. Inactive gene promoters are methylated at certain cytosine residues, resulting in the formation of methylcytosine, which stabilizes nucleosomes while blocking transcription factor binding. The lack of methylation of the HSD17B1 gene in PCOS demonstrates that there is no methylation activity inhibiting HSD17B1 expression. The DNA methylation of HSD17B1 on the cell granulose in PCOS is rarely discussed in the literature, making it difficult to compare. The only one the literature demonstrates and has the same outcome: Wang *et al*. (2014), DNA in ovarian tissue is hypomethylated.

All processes are carried out in accordance with the drawing instructions. Design methylation and unmethylation primers using the MethPrimer program; the biggest island is CpG 874 bp, starting from 1306-2179 bp. The amplification product region promoter gene HSD17B1 has 11 cytosine-guanin dinucleotides.

mRNA Expression Level of the HSD17B1 Gene

The qPCR study showed that PCOS patients had lower levels of HSD17B1 mRNA expression than did healthy women. By applying the Livak approach, we discovered that PCOS patients had a 0.70-fold lower relative expression value of the HSD17B1 gene than did healthy women (Fig. 3). The difference was statistically significant (p = 0.003), according to the Mann-Whitney test statistical analysis of nonparametric data. At p<0.05.

Human granulosa cells in the ovaries and human placenta syncytiotrophoblasts both use the human HSD17B1 enzyme in glandular E2 production. The enzyme's enzymatic properties and expression in these cells indicate a central role in E2 production. High expression in the human placenta is also indicative of its association with E2 production. HSD17B1 is expressed in various peripheral sex steroid target tissues, It supplies extremely potent ligands for estrogen receptors. Human disorders that are endocrine-dependent have been linked to elevated HSD17B1 expression (Hakkarainen *et al*., 2015).

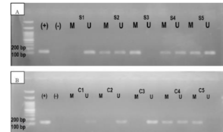

Fig. 1: Electrophoresis HSD17B1 gene

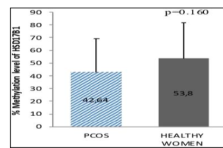

Fig. 2: Methylation percentage of the HSD17B1 gene

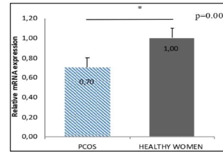

Fig. 3: mRNA expression HSD17B1 gene

According to the research, HSD17B1 controls the availability of estrogen ligands for estrogen receptors in theca cells, which is essential for balanced steroidogenesis in the ovaries. The function of HSD17B1 in luteinization and the onset of pregnancy (Hakkarainen *et al*., 2015) As a result, HSD17Bs are thought to be interesting targets for medications designed to reduce the amount of estrogen present in peripheral estrogen target tissues (Hakkarainen *et al*., 2015).

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Serum E2 Levels in PCOS Patients and Healthy Women

Compared to women in good health, the average E2 serum level in PCOS patients was 25.78 pg/mL. Using an independent t-test, the difference in E2 serum levels was shown to be statistically significant (p = 0.009) p<0.05.

Clinical laboratories test serum estradiol in women to assess ovarian activity. Blood tests for estradiol quantify the level of the hormone. It is used to assess the placenta, adrenal glands and ovaries' overall health. It can identify estrogen in women going through menopause, hypoestrogenicity, amenorrhea, or other menstrual disorders. Estradiol levels rise with follicular development (peak: 200 pg/mL) and are typically less than 50 pg/mL during menstruation in a normal menstrual cycle. In PCOS patients, low estradiol levels were found when compared to healthy women due to the disruption of the work of the genes involved in steredogenesis, so conversion into estradiols is disrupted, which ends with little or inadequate estradiol circulating in the blood (Homer *et al*., 2017).

Correlation Between DNA Methylation and mRNA Expression of the HSD17B1 *Gene*

DNA methylation levels and mRNA expression in PCOS patients did not significantly correlate, according to the correlation analysis utilizing Pearson's correlation test (p = 0.076) (p<0.01) (2-tailed).

The most significant epigenetic fingerprint seen in the genomes of higher eukaryotes is the methylation of DNA at the cytosine ring's position (C-5). Depending on where DNA methylation occurs in an area, it might operate as a limiting or activating mark for gene expression. DNA methylation can thus, through interaction with histone modifiers, either prevent the transcription apparatus from binding or create an environment that is favorable to transcription.

The methylation of DNA at the location of the cytosine ring (C-5) is the most notable epigenetic imprint observed in the genomes of higher eukaryotes. DNA methylation may function as a limiting or activating mark for gene expression, depending on where it happens in the region. Therefore, DNA methylation may either inhibit the transcription apparatus from binding or promote transcription by interacting with histone modifiers.

Studies reveal that favorable correlations between DNA methylation and gene transcription are rare. A study of DNA methylation and gene expression revealed that certain genes were hypomethylated and up-regulated, hypermethylated and down-regulated, hypomethylated and up-regulated, and hypermethylated and down-regulated. A few of these differences were connected to the promoter regions' methylation patterns. may impact methylation changes and transcription factor binding in gene coding regions, which can modulate gene expression through alternative splicing mechanisms even if they do not directly control gene transcription (Hakkarainen *et al*., 2015).

In contrast to our findings, mRNA expression was down-regulated. Wang *et al*. (2014), on the other hand, utilized ovarian tissues as a sample, whereas our investigations employed granulosa cells. The change in the same gene may be caused by the various environmental and lifestyle variables that we know have an impact on PCOS etiology.

Correlation Between the mRNA Expression of the HSD17B1 Gene and Serumm E2 Levels

The substantial (p = 0.020) association between the blood E2 levels and the mRNA expression of the HSD17B1 gene was demonstrated using the Spearman's Rho correlation test at p<0.05.

HSD17B1 genes that are missing or deficient cause downregulation in mRNA genes, which then create E2 that is insufficient for the formation of the estrogen enzyme in the ovaries; this occurrence was demonstrated in this study. An indicator of ovarian activity is serum E2. It is used to assess the placenta, adrenal glands, and ovaries in order to identify hypoestrogenicity and menopause, as well as to detect estrogen in women who have amenorrhea or menstrual disorder. When compared to women in good health, PCOS patients have lower E2 levels because the normal actions of genes involved in steredogenesis are disturbed, which leads to insufficient or inadequate amounts of E2 circulating in the blood.

Electrophoresis of the MSP product of the HSD17B1 gene in granulosa cells (Fig. 1A) 5 PCOS patients (Fig. 1B) and 5 healthy women as controls (+) = positive control; (-) = negative control; M = methylated; U = unmethylated; S1-S5 = sample 1-5 (sample = PCOS group); C1-C5 = control 1-5 (control = healthy women group). The methylated product size was 126 bp, whereas the unmethylated product size was 124 bp.

Methylation percentage of the HSD17B1 gene in granulosa cells from PCOS patients and healthy women Methylation of the HSD17B1 gene in PCOS patients was 42.64% and in healthy women, it was 53.80%. and the statistical analysis of parametric data by the Mann-Whitney test, no significance was found between the two groups (p = 0.160).

Through the Livak method, the relative expression value of the HSD17B1 gene in PCOS patients was down-regulated by 0.70-fold compared with that in healthy women (1.00-fold) (n = 30 in PCOS patients; n = 30 in healthy women). According to the statistical analysis of nonparametric data by the Mann-Whitney test, the result was significant (p = 0.003).

The reason for such AMH overproduction is unknown; however, evidence suggests that androgens play a role. A favorable relationship was discovered between serum androgens and AMH levels (Dewailly *et al*., 2016). It is also related to intrinsic granulosa cell dysregulation, which results in an increase in AMH. It is also caused by intrinsic granulosa cell dysregulation, which has been linked to an increase in AMH receptor type II (Pierre *et al*., 2017; Alebić *et al*., 2015). Certain papers state that gonadotropins, especially FSH, decrease the production of AMH in the blood. Conversely, it explained how FSH stimulates the expression of AMH in both normal and polycystic ovaries (Alebić *et al*., 2015). The recent revelation that E2 inhibits the generation of AMH through the estrogen receptor ß may provide a resolution to this controversial subject. AMH in small antral follicles may be directly increased by FSH.

FSH, on the other hand, can suppress AMH expression by boosting E2 synthesis in bigger follicles due to the negative feedback of E2 (Grynberg *et al*., 2012). In PCOS women, this process is disrupted, since they do not have a corresponding increase in E2 levels. AMH expression and levels in follicular fluid are lower in gonadotropin-dependent follicles in women who are normally ovulatory, but not in PCOS patients (Kristensen *et al*., 2019).

The ovarian reserve may be predicted using the serum AMH value, which represents the quantity and quality of follicular deposits in the ovary. Antral follicle count on ultrasonography, testosterone levels, and ovarian volume are all connected to serum AMH levels. Serum Patients with PCOS have serum AMH levels that are 2-3 times higher than those of healthy women. According to the findings of the study by Sehsu and Dewailly *et al*., The investigation conducted by Sehsu and Dewailly *et al*. revealed that AMH levels may be utilized as a predictor in the diagnosis of PCOS as an alternative to analyzing polycystic ovary pictures and the incidence of PCOS increases as AMH levels rise (Harzif *et al*., 2023).

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Wiweko *et al*. (2014) discovered that people with high AMH levels were 9 times more likely to have PCOS, with the AMH cutoff value as a predictor of PCOS being 4.45 ng/mL. The AMH concentration (6.49 ng/mL) in PCOS participants was above the AMH cut threshold in this investigation (Wiweko *et al*., 2014).

LH Analysis

Studies have shown that blood levels of Luteinizing Hormone (LH) were greater in PCOS patients than in the general population. Despite the lack of a statistically significant difference in this study, LH levels were greater in PCOS individuals. Serum LH levels have been shown to be high in 40-60% of PCOS patients (Yu *et al*., 2015).

LH production is frequently elevated in PCOS patients. High testosterone levels, together with low FSH levels, contribute to poor ovum formation and anovulation. When the menstrual cycle begins, LH levels in PCOS patients are frequently elevated. LH is also more abundant than FSH. Ovulation does not occur without the LH surge and menstruation is irregular. LH hypersecretion is a defining feature of PCOS. This increase in LH production is assumed to be caused by an increase in hypothalamic GnRH pulse frequency. Increased androgen production by theca cells in the ovaries is facilitated by a surge in LH. Excess LH is induced by a fast pulse of release (GnRH) in the hypothalamus, which leads to a dominance of LH hormone output in the pituitary gland. Ovulation abnormalities in PCOS are hypothesized to be caused by elevated LH levels and reduced FSH levels. Normal folliculogenesis is aided by the hormone FSH, which controls follicular development and generates a dominant follicle ready for ovulation. Insulin indirectly increases inhibitory secretion, which suppresses FSH.

Folliculogenesis is significantly impacted by this. In PCOS patients, hypersecretion of LH and insulin results in early luteinization and stops the maturation process in ovarian follicles. Ovarian follicles have polycystic ovarian morphology when their maturation process is interrupted during development, leading to an increase in follicle count. PCOS patients have an increase in theca cells lining their ovaries, which produces androgens (Yu *et al*., 2015).

This leads to premature luteinization of granulosa cells and hyperstimulation of theca cells when paired with the elevated LH levels associated with PCOS. Furthermore, after LH stimulation, PCOS women's granulosa cells expressed more AMH than did those of normal ovulatory women (Alebić *et al*., 2015; 2018; Pierre *et al*., 2017).

FSH Analysis

The average FSH level in patients without PCOS was 7.36 mIU/mL, whereas it was 6.18 mIU/mL in PCOS participants. Despite the fact that the FSH levels were statistically different, both healthy women and PCOS women had normal levels. FSH levels between 3 and 10 mIU/mL are considered normal, whereas values greater than 12 mIU/mL suggest decreased ovarian reserves. More than 25 mIU/mL is associated with ovarian failure and is seen in postmenopausal women (Harzif *et al*., 2023).

The pituitary gland synthesizes 55-57% more LH than FSH due to abnormal control of hypothalamic GnRH production. The serum LH concentration rises, the FSH level falls and the LH/FSH ratio rises. The abnormal dynamic LH expenditure, which is indicated by an increase in the frequency and amplitude of LH pulses, is what is responsible for this elevation in blood LH levels. An increase in GnRH pulse frequency, a persistent negative feedback effect of estrone concentration, and a little increase in inhibin cause FSH

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levels to decline (Yu *et al*., 2015). Rather than fluctuating cyclically as it does in ovulating women, the LH pulse frequency in PCOS women is very constant at around one beat per hour. The same rise in hypothalamic GnRH pulse frequency that encourages the production of LH rather than FSH is most likely the cause of this trend (Harzif *et al*., 2023).

LH/FSH Ratio Analysis

The LH/FSH ratios of the two groups, which were found to be 0.857:1.073 for PCOS patients, did not significantly vary from one another. The ratio typically has a value of 1. The LH/FSH ratio between the two groups in this investigation was discovered to be 1:1. There was no difference in the LH/FSH ratio between the two groups. This ratio, which is around 1:1, indicates that blood levels of FSH and LH are similar and that neither group's ovarian stereogenesis changes are influenced centrally.

However, in PCOS patients, the LH/FSH ratio rises. In healthy women, the LH/FSH ratio should be between 1 and 2. This ratio becomes reversed in women with polycystic ovarian disorder and it can reach as high as 2 or 3. [51] Abnormalities in the adrenal or hypothalamic-pituitary-ovarian axis have been linked to the etiology of polycystic ovarian disease. An increase in the ratio of LH to FSH release is caused by a change in the gonadotrophin-releasing Hormone (GnRH) secretion rhythm. The abnormal feedback mechanism responsible for the rise in LH production is caused by ovarian estrogen. Because of a high LH/FSH ratio, individuals with polycystic ovarian disease do not ovulate (Saadia, 2020).

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Conclusion

Gene expression can be either activated or inhibited by DNA methylation. DNA methylation can either facilitate or hinder the transcription machinery's ability to bind when combined with histone modifiers. This study found that HSD17B1 does not hinder transcription machinery binding but rather creates a transcription-friendly landscape. Reduced mRNA expression of the HSD17B1 gene in PCOS patients can result in reduced E2 levels, thereby contributing to the etiology of PCOS. HSD17B1 is thought to be an interesting target for medicines aimed at decreasing estrogenic burden in peripheral estrogen targets. In clinical laboratories, serum E2 is tested in women to determine ovarian activity. E2 blood tests determine the quantity of E2 present in the body.

Limitations

There is a lack of knowledge on genetic deterioration factors in the family as well as a history of PCOS in the family.

Since pyrosequencing can detect DNA methylation and allele frequency as well as reveal the genetic code of DNA and identify single nucleotide polymorphisms, insertion-deletions, and other sequence variations, it should be used.

We should examine the FSHR gene's DNA methylation because damage methylated or not expressed in this gene can affect the receptor's ability to bind FSH or activate the signal transduction pathway, inhibit the work of estrogen enzymes, and interfere with ovarian and folliculogenesis, all of which contribute to PCOS in women.

We should examine the DNA methylation of the estrogen receptor gene, even if the low level of E2 in PCOS due to down-regulated HSD17B1 expression is already proven, but we also anticipate that the receptor estrogen's potential may be impaired by DNA methylation.

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Author's Contributions

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