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**Original Research Article**

# To estimate the levels of Vitamin D, LH/FSH ratio and Insulin in women with PCOS and find the correlation of Vitamin D with LH/FSH in PCOS Patients with control

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**Abstract**

**Background**

In women of reproductive age, polycystic ovarian syndrome (PCOS) is a frequent endocrinopathy and a heterogeneous, enigmatic condition that is still not fully understood. It begins to manifest between the ages of 18 and 45, and it may take years for its clinical manifestation to manifest. Vitamin D affects progesterone production in human granulosa cells, anti-müllerian hormone (AMH) signalling, follicle-stimulating hormone sensitivity, and ovarian follicular growth and luteinization as part of its physiological role in reproduction. Increased LH/FSH ratios can occur in ovulatory women with the polycystic morphology, but they may not be seen in a single blood sample. With this context in mind, the current study was conducted to determine and evaluate the biochemical tests in individuals with clinical symptoms of polycystic ovarian syndrome, such as Vitamin D, LH/FSH ratio, and serum insulin levels.

**Aim and Objectives**

1. The objective of this study is to estimate the levels of Vitamin D, LH/FSH ratio and serum insulin in women with PCOS.
2. To find the correlation of LH/FSH, with Vitamin D in PCOS Patients and compare with controls.

**Materials and Methods**

The Biochemistry Department and the Department of Gynaecology at SGT Hospital, Budhera, Gurugram's Faculty of Medicine and Health Sciences, conducted this hospital-based cross-

sectional study. We will use 100 healthy volunteers who are age-matched from the general population as the control group. A signed and informed permission will be obtained from each individual in both groups once they have been fully informed of the study's objectives. Before beginning the sample collection, institutional ethical committee approval will be sought. On the fully automated Biochemistry analyzer MAGLUMI 1000, serum insulin, LH/FSH, and vitamin D levels will be determined using a chemiluminescence-based immunoassay (CLIA).

**Results**

Patients with PCOS had a significantly higher level of a parameter, such as serum insulin, with a p value of < 0.0001. The mean and standard deviation of the LH/FSH ratio were also substantially greater in the cases (1.60±0.59) than in the control groups (2.73±1.06). Statistically speaking, the p value was very significant at< 0.001. It indicates that both groups' LH/FSH levels significantly changed. p value <0.0001 indicates that the level of vitamin D is lower in cases when compared to controls. LH/FSH ratio and Vitamin D have negative correlation.

**Conclusion**

When compared to the normal control, our study observed a low value of Vitamin D and significant increase in insulin and an increase in the LH/FSH ratio. Therefore, a multifaceted strategy is needed to identify and track the PCOD condition while employing the entire hormone profile, allowing for prompt and effective intervention. The measurement of all these hormones in conjunction with radiological correlation can be used to diagnose PCOD and treat this condition at different stages.

**Key Words:** Vitamin D, Insulin, Luteinizing Hormone (LH), Follicle- Stimulating Hormone (FSH)

## 1. INTRODUCTION:

In women of reproductive age, polycystic ovarian syndrome (PCOS) is a frequent endocrinopathy and a heterogeneous, enigmatic condition that is still not fully understood. It begins to manifest between the ages of 18 and 45, and it may take years for its clinical manifestation to manifest. 50% of women seen at infertility clinics and 4% to 22% of all women have polycystic ovarian syndrome (1,2). In 1935, Irving Stein and Michael Leventhal made the first official diagnosis of polycystic ovarian syndrome in a group of patients who had amenorrhea, bilateral polycystic ovaries, and masculinizing changes. According to them, these symptoms could be the result of hormonal stimulation that was most likely caused by the anterior lobe of the pituitary. Even after 70 years, the pathophysiology is still the subject of considerable study and controversy. The underlying cause for its heterogeneity and the emergence of indications and symptoms has not been discovered (3). Low levels of follicle-stimulating hormone, which leads to anovulation, high levels of luteinizing hormone, which causes hyperandrogenism, and insulin resistance are the main characteristics of PCOS. These symptoms can range from simple cystic acne, cephalic hair loss, or mild facial hirsutism to cases of oligo- or amenorrhea, sterility, and severe generalised hirsutism. Traditional Stein-Leventhal syndrome, characterised by hirsutism and amenorrhea. Vitamin D affects progesterone production in human granulosa cells, anti-müllerian hormone (AMH) signalling, follicle-stimulating hormone sensitivity, and ovarian follicular growth and luteinization as part of its physiological role in

reproduction. About 67–85% of women with PCOS have vitamin D insufficiency, with blood values of 25(OH)D 20 ng/ml. (4) Low levels of 25(OH)D may make PCOS symptoms worse, such as insulin resistance, irregular menstruation, infertility, hyperandrogenism, obesity, and an increased risk of cardiovascular illnesses (5,6). A study by Waldstreicher et al. evaluated frequent (every 10 minutes) and extended (12-24 hours) serial blood samples, and the results showed that PCOS patients had a significantly higher frequency and amplitude of LH release compared to controls (7). The higher LH pulse frequency indicates a malfunction in the hypothalamus since it corresponds to an increase in GnRH secretion. Increased LH/FSH ratios can occur in ovulatory women with the polycystic morphology, but they may not be seen in a single blood sample. In light of this, the current study was conducted to determine and evaluate the biochemical tests in the form of Vitamin D, Insulin, LH/FSH ratio, and serum prolactin levels in patients with polycystic ovarian syndrome clinical characteristics.

## 2. MATERIALS AND METHODS:

Faculty of Medicine and Health Sciences, SGT Medical College, Hospital & Research Institute (SGT University), Budhera, Gurugram, Haryana, conducted the current hospital-based observational cross-sectional study. At the Obstetrics and Gynecology OPD of SGT Hospital Budhera, Gurugram, sixty patients between the ages of 18 and 45 who had been diagnosed with "polycystic ovarian syndrome by using Rotterdam criteria" were included in the study. The diagnosis has to meet two requirements:

- 12 follicles with a diameter of 2 to 9 mm are present.
- Each ovary's volume exceeds 10 cm3 when peripheral follicle volume is included.100 age matched healthy volunteers from general population will be taken as control.

Prior to collecting the samples and receiving approval from the institutional ethical committee of SGT University, Gurugram, a signed and informed consent was obtained from each subject after fully explaining the study's goals and methodology. The study excluded patients with any other chronic conditions, patients taking vitamin D medications, pregnant women, and patients with other endocrine disorders. Between the second and third days of the menstrual cycle, 5 ml of venous blood were drawn. Samples were collected in capped vacutainers and centrifuged for 15 minutes at 3500 rpm. On the fully automated Biochemistry analyzer MAGLUMI 1000, serum vitamin D, LH/FSH, and insulin levels will be determined using a chemiluminescence-based immunoassay (CLIA).

### Statistical analysis

SPSS (statistical package for social science) software was used for the statistical analysis. Every bit of information was presented as mean + SD + SE of the mean. A p value of 0.001 or lower was deemed significant. A student t-test was used to compare the data between the two groups. For correlation between two quantitative variables, Pearson's correlation coefficient was used. For non-parametric variables, the chi square test was used.

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## 3. RESULTS:

**Tables:**

| Age group(yrs) | Cases | Control |
|---|---|---|
| 18-22 | 7 | 7 |
| 23-26 | 10 | 10 |
| 27-30 | 25 | 25 |
| 31-34 | 12 | 12 |
| 35-38 | 23 | 23 |
| 39-42 | 16 | 16 |
| 43-46 | 7 | 7 |

Table 1. Distribution of cases and control based on age group

| | Cases | Control | p value |
|---|---|---|---|
| Mean± SD | 32.91±6.71 | 33±6.69 | 0.927 |
| | | | Not significant |

Table 2:- Showing mean and standard deviation of age in cases and controls

| S.NO | Parameters | Mean±SD in cases | Mean ±SD in control | P value |
|---|---|---|---|---|
| 1 | 25OH | 20.21±12.21 | 27.38±6.63 | <0.001 |
| 2 | LH | 16.01±7.06 | 6.99±2.20 | <0.001 |
| 3 | FSH | 6.06±2.28 | 4.72±1.70 | <0.001 |
| 4 | LH/FSH | 2.73±1.06 | 1.60±0.59 | <0.001 |
| 5 | Serum insulin | 19.07±14.68 | 4.05±1.57 | <0.001 |

Table 3 :- Represents mean with SD values of serum 25OH, LH ,FSH, LH/FSH ratio and serum insulin levels among PCOD cases and control groups.

**Figures:**

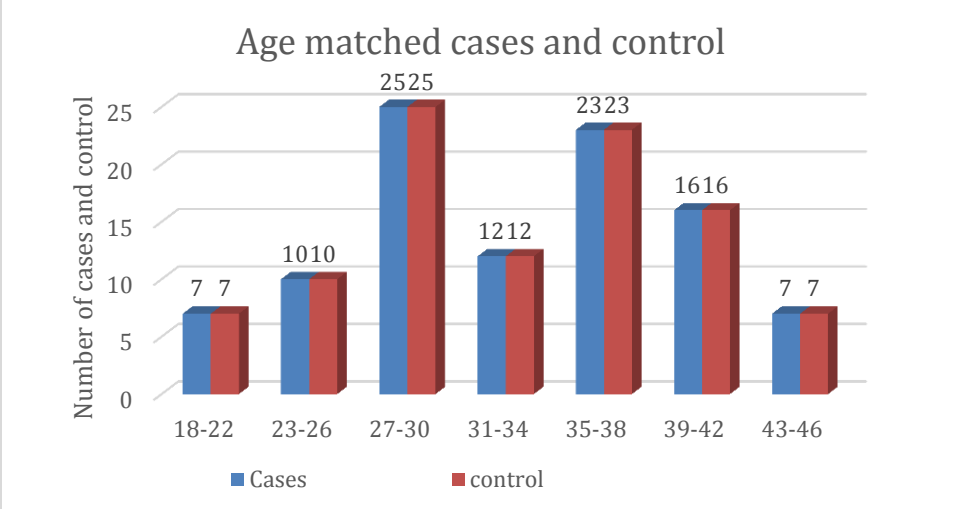


**Fig -1:** Graph showing Distribution of cases and control based on age group

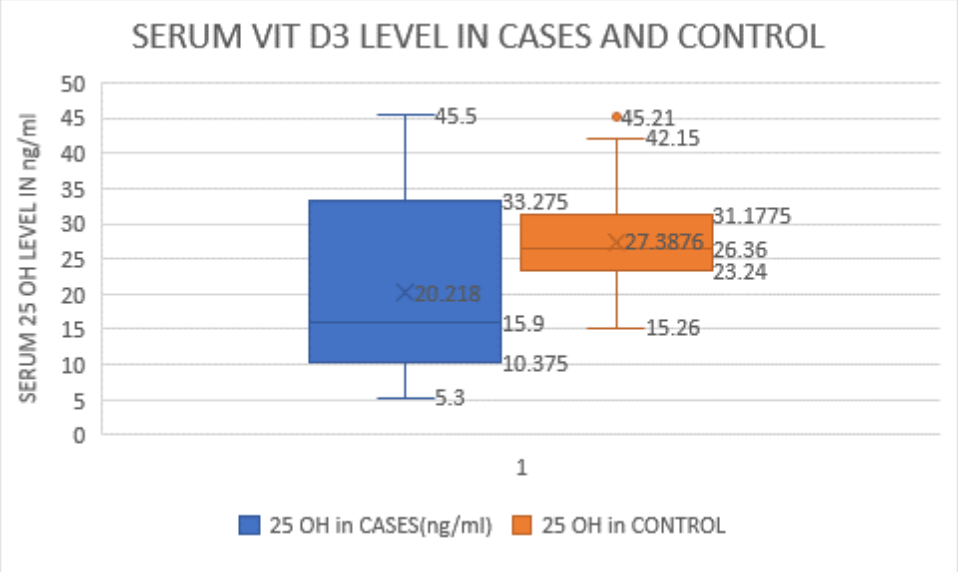


**Fig 2**: Graphical representation of serum vit D level in the form of box and whisker chart

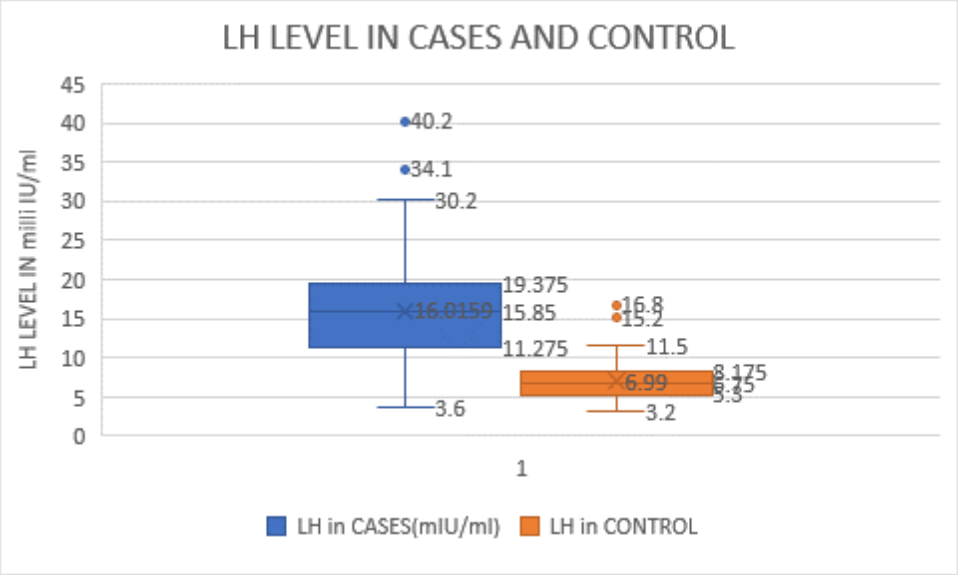


**Fig 3:-**Graphical representation of serum Leutenizing hormone in the form of box and whisker chart

ISSN 2515-8260 Volume 10, Issue 02, 2023

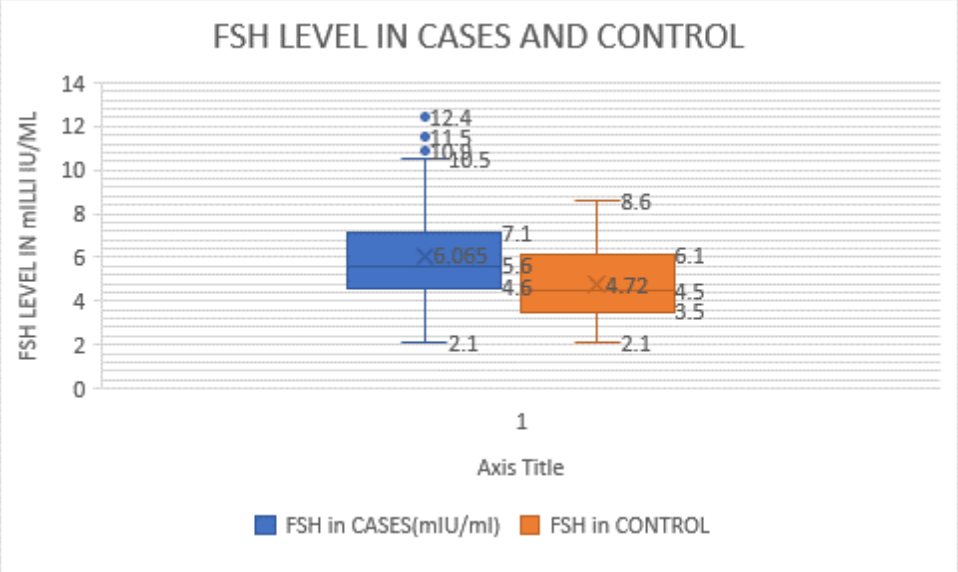


**Fig 4:-**Graphical representation of serum Follicle stimulating Hormone in the form of box and whisker chart

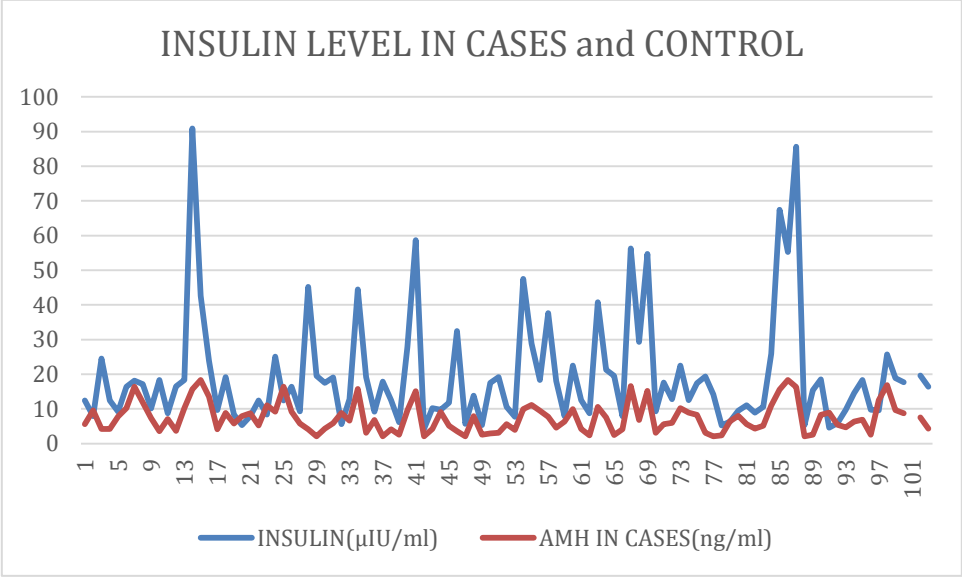


**Fig 5:-**Graphical representation of fasting insulin level in the form of box and whisker chart

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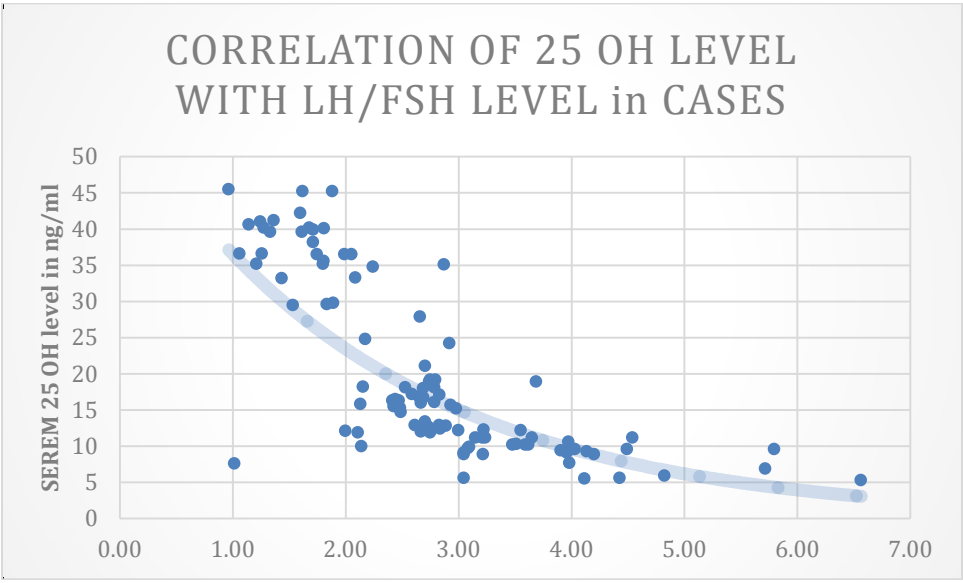


**Fig 6:**- Graph represent correlation between serum 25 hydroxy vit D level and LH/FSH ratio in PCOD cases- here pearson's coefficient noted r= - 0.75, which is negative correlation between these two parameters (LH=leutenizing hormone, FSH= Follicle stimulating hormone and 25 OH= 25 hydroxy vit D level)

This study set out to measure the levels of vitamin D, insulin, LH/FSH ratio, and connect LH/FSH ratio with vitamin D.

**Demographic Parameters:**

In this study, 100 PCOD cases and 100 age-matched control group participants were both included. Table 1 and Figure 1 reveal that the majority of cases in this group, or 25 instances, were in the age range of 27 to 30 years, followed by 23 cases in the 35 to 38 years age range. A minimum of 7 cases, or people between the ages of 18 and 22, and 43 and 46, were involved. There were 16 instances in the 39–42 year old age group, whereas there were 12 and 10 cases in the 31–34 and 23–26 year old age groups, respectively.

In this study, age in years was evaluated, and the mean and standard deviation were calculated as 32.91±6.71 years for cases and 33.66 years for control groups. The p value was recorded as 0.927, denoting the absence of any discernible differences between the case and control groups. This means that the groups in table 2 are age-matched.

In our investigation, the mean and standard deviation of serum 25-hydroxyvitamin D levels in cases were considerably lower than those in control groups, measuring 20.21±12.21 versus 27.38±6.63 respectively. Statistically speaking, the p value was very significant at <0.001. It indicates that there was a significant change in the levels of serum 25-hydroxyvitamin D in both groups. Figure 2 (Table 3).

The mean and standard deviation of the LH/FSH ratio were also substantially greater in the cases (1.600.59) than in the control groups (2.73±1.06). Statistically speaking, the p value was very significant at 0.001. It indicates that both groups' LH/FSH levels significantly changed. Figure 3 (Table 3 and 4)

Additionally, the mean and standard deviation of serum insulin levels were greater in cases (i.e., 19.07±14.68 vs. 4.05±1.57 in control groups). Statistically speaking, the p value was

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very significant at <0.001. It indicates that there was a significant change in both groups' fasting serum insulin levels. (Figure 5 in Table 3).In our study we found a negative correlation between these two parameters (LH=leutenizing hormone, FSH= Follicle stimulating hormone and 25 OH= 25 hydroxy vit D level)having pearson correlation coefficient r =0.-75 shown in (Figure 6)

## 4. DISCUSSION

Women with PCOS diagnosed between the ages of 18 and 45 were the subject of this study. In order to determine the levels of fasting insulin, LH/FSH, and vitamin D in patients with polycystic ovarian syndrome, LH/FSH ratio was performed. According to the Rotterdam European Society of Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine (ASRM) criteria, individuals with polycystic ovarian syndrome were identified in this study.

In present study serum 25 hydoxy vit d level were low in PCOD cases i.e 20.21±12.21 in comparision to control group 27.38±6.63. In study comducted by Kharb et al showed similar results with serum 25 hydroxy vit d level in PCOD cases as 19.29 ± 5.86 has reported vitamin deficiency in 72.8% PCOS women and Kim et al. (8) reported no difference in the level of serum 25- (OH) vitamin D in PCOS women when compared with controls . Whereas, Kumar et al.(9) reported decreased levels of serum 25-OH vitamin D in PCOS women in comparison to controls.

In the current investigation, cases and the control group's respective mean and standard deviation (SD) levels of serum LH were 16.01 ± 7.06 respectively. In a study by Vinay Kr et al. (10) it was observed as 6.99±2.20, which is comparable. The PCod patients and control populations in the study by Budi Wiweko et al. (11) were 10.41±8.12 and 4.37, respectively. Additionally, Shahrzad Zadehmodarres et al. (12) found that the serum LH levels in the patients and control group were 5.96± 2.93 and 5.58± 3.09, respectively. In an Indian study by Nalini Mahajan et al., the cases and control groups were observed as 6.86±4.72 and 4.22±2.26 respectively (13).

The mean±SD level of serum FSH was 6.06±2.28 and 4.72±1.70 in the current study's case and control groups, respectively. which is comparable to a study by Vinay Kr et al (10), where 5.78±2.42 was discovered. However, in the study by Budi Wiweko (11) et al., the numbers for PCOD patients and controls were 5.391.47 and 6.30, respectively. Additionally, Shahrzad Zadehmodarres et alstudy .'s (12) reported serum FSH levels of 4.5±3 1.62 and 6.88 ±5.56 in the patients and control groups, respectively. In an Indian study by Nalini Mahajan et al., the cases and control groups were observed as 4.23±1.29 and 6.68±2.22 respectively (13).

In the current investigation, patients and controls had LH/FSH ratios of 2.73± 1.06 and 1.60± 0.59, respectively. Which is comparable to a case-control study conducted on women with and without PCOS by Hilma Putri Lubis et al. (14) published in 2020, where they found Lh/FSH levels of 2.10± 1.16 and 0.65± 0.31 in the cases and control groups, respectively. In a study by Li Wei Cho et al. (15), the LH/FSH ratio was recorded as 1.2 and 1.6 in the case and control groups, respectively.

In the current investigation, PCOD cases had fasting insulin levels that were higher (19.07±14.68) than control groups (4.05±1.57). Which corelate with the study conducted by

Avin S Jamil et al.(16) and Homeira Rashid et al (17) in which fasting serum insulin level were raised in comparision to control groups.

## 5. CONCLUSION

A common endocrine disease called polycystic ovary syndrome affects 5–10% of women who are pregnant or premenopausal. Chronic anovulation (oligoovulation or amenorrhea) and hyperandrogenism are its defining features (hirsutism). Along with symptoms of hyperandrogenism and onvulatory dysfunction, PCOS is also linked to obesity and insulin resistance. These people are more likely to experience aberrant glucose metabolism, which leads to the onset of insulin resistance. Hyperinsulinemia, which is brought on by insulin resistance, helps to boost the production of androgens. The delay in follicular maturation has been linked to LH's excessive and premature impact on PCOS granulosa cells. The increase in ovarian androgen levels brought on by LH may potentially be the reason of this. An ovulation in PCOS has been linked to the refractoriness of granulosa cells to FSH action, in addition to the absence of the intercycle FSH peak and other factors. In our investigation, we measured the LH/FSH ratio in 100 samples and discovered a discernible increase in the ratio in PCOD instances.

In this investigation, we discovered that PCod patients had significantly higher serum insulin and LH/FSH ratios than normal controls. While vitamin D levels are low compared to controls. Additionally, we observed a bad association between vitamin D and LH/FSH.

Therefore, a multifaceted strategy is needed to identify and track the PCOD condition while employing the entire hormone profile, allowing for prompt and effective intervention. The measurement of all these hormones in conjunction with radiological correlation can be used to diagnose PCOD and treat this condition at different stages.

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