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A study on LH/FSH ratio in polycystic ovarian syndrome patients

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ABSTRACT

Introduction

A high percentage about 55–75 % of women with PCOS have an elevated LH/FSH ratio presumably due to high levels of Luteinizing harmone (LH) rather than reduced production of Follicular stimulating hormone (FSH).

Aim/objective

The present study aimed to measure the LH concentrations in accurately timed samples in PCOS patients and normal controls and to assess the importance of LH/FSH ratio as a diagnostic tool for PCOS and to find a correlation or association among LH/FSH ratio, BMI in PCOS patients.

Materials and Method

It's a Case-control study which includes 46 subjects with cases (n=23) and controls (n=23). Cases include 23 female patients between 17-29 years with PCOS who attended GITAM Institute of Medical Sciences and Research Hospital (GIMSR), Visakhapatnam, Andhra Pradesh, India. Serum LH and FSH is measured by Electrochemiluminescent immunoassay (ECLIA) method in the present study.

Results

By performing a paired 't' test analysis and correlation analysis between cases and controls for the variables like FSH, LH, FSH/LH and BMI, the results were significant for LH (p=0.008) and insignificant for FSH, LH/FSH and BMI p>0.05. By performing a paired t test analysis, the results showed LH/FSH ratio and BMI strongly influences and play a significant role in PCOS patients.

Conclusion

Elevated LH concentrations are found in a large majority of PCOS patients, when measured at the appropriate time. Due to the high positive predictive value, LH could be used as an additional diagnostic test to diagnose PCOS. Further studies are required to identify the importance of elevated LH in PCOS patients.

Keywords: PCOS, Luteinizing Hormone, Follicular Stimulating Hormone, BMI, Infertility

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is defined as the most frequent endocrine problem in women of reproductive age, which increase the risk of infertility. Prevalence is estimated to about 12–21% in women of reproductive age. The clinical signs in a PCOS female are oligo or anovulation, hirsutism, acne, and polycystic ovaries on ultrasound. These symptoms in PCOS include unwanted hair growth, dark patches of skin, acne, weight gain, and irregular bleeding which are due to hormonal imbalance that can affect women.[1]

Women with PCOS have a wide range of clinical symptoms. Menstrual abnormalities, clinical symptoms of hyperandrogenism, and infertility are common complaints among women with PCOS. Oligomenorrhea, amenorrhea, and extended unpredictable menstrual flow are all prevalent menstrual irregularities in PCOS. 30% of women with PCOS, on the other hand, have normal menses. Approximately 85%–90% of women with oligomenorrhea have PCOS while 30%–40% of women with amenorrhea will have PCOS. Infertility affects 40% of women with PCOS.[2-5]

Anovulatory infertility is most commonly caused by PCOS. PCOS affects 90 percent to 95 percent of anovulatory women who visit infertility clinics. The number of primordial follicles in women with PCOS is normal, but the number of main and secondary follicles is much higher. When elements involved in normal follicular formation are disrupted, follicular growth is arrested. When follicles attain a diameter of 4–8 mm, however, follicular growth is stopped due to disruptions in components essential in normal follicular development. Ovulation does not occur because a dominant follicle does not form. PCOS is a heritable ailment, with a family history of the condition being a risk factor. According to a study, 38% of PCOS patients were overweight (BMI >25 kg/m2).[6]

Obesity was found to be connected with a higher risk of hirsutism and menstrual cycle irregularities. The onset of PCOS clinical characteristics is frequently preceded by a history of weight gain. Furthermore, diet-induced weight loss has a significant impact on ovulation because it lowers blood testosterone levels and improves ovulation. A higher prevalence of PCOS has been linked to a number of health issues. PCOS patients have an android body type, with a waist-to-hip ratio of more than 0.8. In women with PCOS, living a healthy lifestyle has been demonstrated to reduce body weight, belly fat, testosterone, insulin resistance, and hirsutism.[7]

Extraovarian factors include a variety of endocrine, paracrine, and metabolic changes that can affect folliculogenesis and oocyte development by producing anomalies in the follicular microenvironment. Follicular stimulating hormone (FSH) deficiency, hypersecretion of luteinizing hormone (LH), ovarian or adrenal hyperandrogenemia, and insulin resistance are all examples of these changes. A substantial majority of women with PCOS, roughly 55–75 percent, have an elevated LH/FSH ratio, which is likely owing to excessive amounts of LH rather than decreased FSH production. PCOS patients, on the other hand, collect antral follicles (2–8 mm) that differentiate early and undergo premature growth arrest (because to LH and FSH concentrations that are greater and lower than normal, respectively). Hypersecretion of LH in these women could enhance granulosa cell luteinization and contribute to antral follicle growth arrest.[8]

Even though the illness is linked with a variety of symptoms, the diagnosis is made using Rotterdam consensus criteria. Furthermore, PCOS patients may have excessive LH, a high LH/FSH ratio (>2), and normal FSH. This, however, isn't one of the diagnostic criteria. For a long time, an LH/FSH ratio greater than two has been regarded the "gold standard" in PCOS diagnosis. LH has been used as a diagnostic test to distinguish between women with PCOS and healthy controls in a number of studies, with the LH/FSH ratio in particular being proven to be predictive. A single test fails to identify an elevated LH/FSH ratio due to the pulsatile nature of their release.[8,9]

The present study aimed to measure the LH concentrations in adequately timed samples in PCOS

patients and normal controls and to assess the importance of LH/FSH ratio as a diagnostic tool for PCOS and to find a correlation or association among LH/FSH ratio, BMI in PCOS patients.

METHODS AND MATERIALS

It's a Case-control study which includes 46 subjects with cases (n=23) and controls (n=23). Cases include 23 female patients between 17-29 years with PCOS who attended GITAM Institute of Medical Sciences and Research Hospital (GIMSR), Visakhapatnam, Andhra Pradesh, India. Controls include 23 age matched apparently healthy female participants.

This study included female patients of age group ranging from 17-29 years and who are newly diagnosed to have PCOS based on Rotterdam criteria and are not on treatment. Also, this study excluded patients with diabetes mellitus, pregnant and lactating women, patients on any medications that act on hypothalamic-pituitary-gonadal axis such as contraceptives, hormonal therapy (estrogens or progestins), endocrine therapy for breast cancer, GnRH analogues etc., and drugs that alter prolactin levels like anti-psychiatric drugs. Patients with hyperandrogenemia or oligomenorrhoea due to other endocrine causes like androgen producing tumours, adrenal hyperplasia, prolactinomas, premature ovarian failure, primary hypothalamic amenorrhoea, conditions that produce symptoms similar to PCOS like Cushing's syndrome, hypothyroidism etc. are excluded from the study.

Study Design

Institutional Ethics Committee (IEC) clearance approval was obtained before the start of the study. Each participant was explained about the details of the study and informed consent obtained. No incentives were given to the participant for being a part of the study. The study was conducted for a period of 3 months between March to April 2021. Participants for the study were selected by taking detailed history and by Ultrasound abdomen findings. Subjects categorised into two groups as cases and controls based on Rotterdam criteria of diagnosis of PCOS.

5ml of blood sample was withdrawn from participants after overnight fasting of 10-12 hours. Blood samples collected from both cases and controls were centrifuged at 3000 rpm for 15 min. Serum was separated and stored at -20^0 C until analysis. Sample test tubes were appropriately labeled and all specimens were considered as potentially infectious. Hemolysed samples or samples with insufficient volumes were rejected. Serum LH and FSH concentrations were estimated in the serum samples by Electrochemiluminescent immunoassay (ECLIA) in ROCHE cobas e411 instrument.

Measurement of LH

Serum LH hormone concentration was measured using the Cobas e411 analyzer (Roche Diagnostics, Mannheim, Germany) based on an electrochemiluminescent immunoassay in accordance with the manufacturer's protocol. It employs two monoclonal antibodies specifically directed against human LH. The two specific antibodies used recognize particular conformations, with the biotinylated antibodies detecting an epitope constructed from both subunits whereas the antibody with the ruthenium complex label detects an epitope from the β-subunit. As a result, the Elecsys LH assay shows negligible cross-reactivity with FSH, TSH, hCG, hGH, and hPL. The test was based on the Sandwich principle and the total duration for assay took 18 minutes.

1st Incubation

20 µL of sample, a biotinylated monoclonal LH-specific antibody, and a monoclonal LH-specific antibody labeled with a ruthenium complex form a sandwich complex.

2nd Incubation

After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a

photomultiplier. Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.[10]

Limits and Measuring Range

Measuring range are between 0.1 to 200 mIU/ml. The lower detection limit is 0.1 mIU/ml. Values below the lower detection limit are reported as < 0.1 mIU/ml. Values above the measuring range are reported as > 200 mIU/ml.

Table 1 Expected Values Based on Studies with the Elecsys LH Assay

Gender	Phases	Values
Males	Adult	1.2 – 7.8 mIU/mL
Females	Follicular phase	1.7 – 15.0 mIU/mL
	Mid cycle	21.9 – 56.6 mIU/mL
	Luteal	0.6 – 16.3 mIU/mL
	Post menopausal	14.2 – 52.3 mIU/mL

Measurement of FSH

Serum FSH was measured by Electrochemiluminescent immunoassay (ECLIA). The Elecsys FSH assay employs two different monoclonal antibodies specifically directed against human FSH. Cross-reactivity with LH, TSH, hCG, hGH, and hPL is negligible. The test was based on the Sandwich principle and the total duration for assay take 18 minutes.

1st Incubation

40 µL of sample, a biotinylated monoclonal FSH-specific antibody, and a monoclonal FSH-specific antibody labeled with a ruthenium complexa) form a sandwich complex.

2nd Incubation

After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode

Limits and Measuring Range

Measuring ranges are between 0.1 to 200 mIU/ml. The lower detection limit is 0.1mIU/ml. Values below the lower detection limit are reported as< 0.1mIU/ml. Values above the measuring range are reported as> 200 mIU/ml. Table 1 and Table 2 showed the expected values of LH and LSH in both genders.

Table 2 Expected Values Based on Studies with the Elecsys FSH Assay

Gender	Phases	Values
Males	Adult	1.4- 15.4 mIU/mL
Females	Follicular phase	1.4-9.9 mIU/mL
	Mid cycle	0.2-17.2 mIU/mL
	Luteal	1.1- 9.2 mIU/mL
	Post menopausal	19.3-100.6 mIU/mL

RESULTS

The data for the demographic variables age, BMI and study variables LH, FSH and LH/FSH ratio was represented in the MS-Excel sheet and the data was analyzed through Paired t-test analysis and Pearson's

correlation analysis using SPSS software 24.0 Version. The mean age group of the study was 23.7 years.

From the Table 3 it was showed that, there was no marked increase in the values of FSH between cases and control samples hence the results were insignificant (P>0.05), on the other hand though there was a difference in the mean values of LH between cases and controls it was statistically insignificant (P>0.05). It was also observed that, there was a significant (P<0.05) rise in the LH/FSH ratio and BMI between the case and control samples. From the 2-tailed paired t test analysis, the results showed LH/FSH ratio and BMI strongly influences and play a significant role in PCOS patients.

Table 3 Paired T Test Analysis for the Study Variables

Parameters	Mean	Std. Deviation	Std. Error Mean	t-value	P value
FSH (CASE/CONTROL)	5.5465	2.23174	.46535	-.019	0.985
	5.5600	2.01599	.42036		
LH (CASE/CONTROL)	10.6222	5.78402	1.20605	1.773	0.090
	7.5570	3.58569	.74767		
LH/FSH Ratio (CASE/CONTROL)	2.0013	.75613	.15766	3.535	0.002
	1.3683	.40178	.08378		
BMI (CASE/CONTROL)	24.9739	4.04882	.84424	4.110	0.001
	20.7217	2.23341	.46570		

By performing a Pearson's correlation analysis (Table 4) between cases and controls for the variables like FSH, LH, FSH/LH and BMI, the results were significant for LH (P=0.008) and insignificant for FSH, LH/FSH and BMI P>0.05. However all these four factors were negatively correlated to PCOS.

Table 4 Correlation Analysis

Parameters	Correlation	Sig.
FSH (CASE/CONTROL)	-.334	.120
LH (CASE/CONTROL)	-.542	.008
(LH/FSH RATIO) CASE/CONTROL	-.008	.973
BMI (CASE/CONTROL)	-.179	.413

DISCUSSION

Polycystic ovarian syndrome is the topic of ongoing research into its pathophysiology, diagnostic tools, and treatment options. Insulin resistance is significantly linked to obesity of the androgenic kind (abdominal).[11] In our study, the majority of patients in the cases group had a high BMI, with a mean score of 24.9, putting individuals in the obese category. The LH/FSH ratio was still considered the "gold standard" for PCOS diagnosis, and the coexistence of insulin resistance and hyperinsulinemia was only beginning to be recognized as a possible pathogenic factor. Overproduction of LH and, as a result, an increased LH/FSH ratio are no longer considered to be a defining feature of all PCOS patients. In this study, PCOS patients had an elevated LH/FSH ratio, with a ratio of 2.0013 in cases that were higher than the control group. According to some research, the prevalence of an increased LH/FSH ratio is as high as 94%.[12] It is now thought that people with insulin resistance and hyperinsulinemia are more likely to have increased LH levels than those without hyperinsulinemia.[8,9] Thus, the most severe clinical symptoms and greater androgen concentration is seen in women with hyperinsulinemia and overproduction of LH. Hirsutism of greater severity was observed in a group of women with hyperinsulinemia and LH/FSH ratio > 2. It could be hypothesized that the excess of LH may reduce the influence of insulin on SHBG production.[13]

This study assessed the prevalence of elevated LH concentrations in adequately timed samples and its importance as a tool in the diagnosis of PCOS. In this study, by performing a 2-tailed paired t test analysis, the results showed LH/FSH ratio and BMI strongly influences and play a significant role in PCOS patients. High serum LH concentrations are often found in PCOS patients, and this is an inextricable feature of PCOS. Besides causing ovulation and luteinization, LH is known to stimulate ovarian androgen production [14], and is seen as one of the main causes for hyperandrogenism in PCOS patients. The role of elevated LH concentrations in the pathogenesis of PCOS is unclear, and the question remains if it is part of the cause of PCOS or is a result of the endocrine and cycle disturbances. The more severe forms of PCOS seem to be associated with higher LH concentrations. Previous studies showed that follicle number and ovarian volume are positively related to LH and more severe cycle disturbances and an increased infertility rate were found with increasing LH concentrations in PCOS patients.[15-19] A more pronounced decrease in LH concentrations is also seen in PCOS women responding to ovarian surgery to induce ovulation than in non-responders.[20,21]

CONCLUSION

Proper diagnosis and management of PCOS is essential as PCOS has many potential metabolic and cardiovascular risks if not managed appropriately focused on individual symptoms, not the syndrome itself. However, as the understanding of the pathophysiology of PCOS improves, so does the treatment. Although treatment should be individualized, it should also focus on all metabolic consequences and decreasing future complications. In conclusion, elevated LH concentrations are found in a large majority of PCOS patients, when measured at the appropriate time. Due to the high positive predictive value, LH could be used as an additional diagnostic test to diagnose PCOS. Further studies are required to identify the importance of elevated LH in PCOS patients.

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