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Relationship between Luteinizing Hormone/Follicle Stimulating Hormone Ratio and Body Mass Index in Women with Polycystic Ovarian Syndrome

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ABSTRACT

Background: The use of luteinizing hormone to follicle-stimulating hormone (LH/FSH) ratio in the diagnosis of polycystic ovarian syndrome (PCOS) is controversial. **Objective:** To correlate LH/FSH ratio with body mass index (BMI) among women with PCOS, and the possible use of LH/FSH ratio as predictive diagnostic tool of PCOS. **Materials and Methods:** Serum LH and FSH were assayed by the Enzyme-linked Immunosorbent assay (ELISA) technique using reagents supplied by Monobind, USA. Students-t-test was used to compare the mean values of LH, FSH, and LH/FSH ratio between cases and controls. Pearson correlation was used to associate a measured variable with age and body mass index (BMI). **Results:** The mean LH and FSH levels were significantly higher ($p<0.001$) among the infertile women with PCOS when compared to the fertile controls. The LH/FSH ratio was significantly higher ($p<0.001$) among women with PCOS than control subjects. The serum LH and FSH correlated with age ($r=0.318, p<0.05$; $r=0.320$, $p<0.05$) respectively among women with PCOS. There was a significant correlation between LH and BMI but not between FSH and BMI. On the other hand, LH/FSH ratio correlated with both age and BMI ($r=0.319$, $p<0.05$; $r=0.309$, $p<0.05$) among women with PCOS. **Conclusion:** The LH: FSH ratio may be considered as a diagnostic index of PCOS in women with infertility. Further study involving a large population is suggested to ascertain the diagnostic utility of the LH/FSH ratio among Nigeria women with PCOS.

Keywords: Female, polycystic ovarian syndrome, body mass index, luteinizing hormone, follicle stimulating hormone.

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This work was conducted and approved in collaboration between all the authors, who takes responsibility for its accuracy and integrity. MA designed the study; EO, RO sourced for funding; MA, EO wrote the protocol; EO, RO contributed in literature search; EO, RO did the experiments; RO, MA did statistical analysis; RO drafted the manuscript; MA supervised the study; MA Wrote the final manuscript; MA proofread the final manuscript for publication.
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INTRODUCTION

The polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age and is a complex condition that is most often characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovaries as well as some medical features that may include obesity, hirsutism, alopecia, acne, irregular menses, infertility and high blood pressure (1). About 60% of patients with PCOS are overweight or obese (2). Loss of weight and dietary management is associated with improved spontaneous ovulation in women with PCOS likely due to improved insulin sensitivity (3). Diagnosis of PCOS is made when a woman of reproductive age presents with androgen excess, ovulation disorder, and polycystic ovaries in the absence of other illnesses that mimic PCOS. The development of PCOS has been linked to altered luteinizing hormone (LH) action. The diagnosis of PCOS has varied over the years and included requirements of anovulation or polycystic ovaries, with androgen excess. Subjects with PCOS do present with different signs and symptoms, which may make it difficult for the accurate evaluation of the condition. Diagnosis of PCOS is currently based on the Rotterdam European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) consensus statement on PCOS (4). Diagnosis is made based on the finding of at least two of the following features; Oligo-anovulation, hyperandrogenism, and polycystic ovaries by ultrasound (4).

During the reproductive age, it may lead to anovulatory infertility and the condition has been associated with increased prevalence of miscarriage, gestational diabetes, and preeclampsia (5). Early diagnosis is crucial to enable close follow-up and, in an attempt, to reduce the risk of associated complications (6). Although the prevalence of PCOS is similar in most countries of the world, ethnic factors have been considered to influence the phenotypic manifestations of the disorder. It is believed that the existence of different environmental factors such as diet, physical activity, and lifestyle might contribute to the variability (6,7).

Obesity may be a potential barrier to effective fertility treatment in women with PCOS. The association of PCOS and successful assisted reproductive outcome has been the subject of observational studies. It has been reported that obese women with PCOS had less number of retrieved oocytes and clinical pregnancy rates with a higher rate of abortions (8). On the other hand, some authors have suggested that obese and non-obese PCOS patients revealed no significant differences in assisted reproduction methods (9). The association of obesity with PCOs has been reported in 30-75% of women elsewhere (10). More recently, however, it was stated that whether or not in PCOS, pregnancy attainment is affected by body mass index (BMI). Obese women have been observed to experience lesser clinical pregnancy with an increased miscarriage rate after obtaining infertility management (1).

The suggestion of some authors to include the LH/FSH ratio as a predictive diagnostic tool for PCOS has been controversial (11,12). Whereas some have reported that LH/FSH ratio greater than 2 can be accepted as abnormal and be used in the diagnosis of a subgroup of individuals with PCOS (11), others have expressed concerns about the clinical utility in clinical practice (13). A more recent study has demonstrated that LH: FSH ratio is a valuable diagnostic tool in evaluating Nigerian women with PCOS (12). Some authors (14) reported that not every subject with PCOS has hormonal and biochemical alterations suggestive of

the disease. It is important to know if there is a correlation between LH/FSH ratio and PCOS among Nigerian women since different environmental factors contribute to the variability (6,7). This study, therefore, seeks to correlate LH/FSH ratio with age and BMI among women with PCOS and to determine whether the ratio is higher among the study group than controls.

MATERIALS AND METHODS

Study Population:

The study was a case-control study design conducted among 40 women with PCOS, mean age 32.35±2.00years, mean BMI 31.5±0.5 kg/m^2 and 40 healthy women of proven fertility with mean age 33.31± 0.20 years, mean BMI 30.2±0.4kg/m^2 and without any evidence of PCOS. The diagnosis of PCOS was made by the attending physicians according to the Rotterdam consensus criteria (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2003). The diagnosis was made based on the finding of at least 2 out of the following 3 features (i) Oligo- and/or anovulation; (ii) Clinical and/or biochemical evidence of hyperandrogenism; and (iii) Transvaginal ultrasound scan morphology of PCO with 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume >10 mL (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group,2003). The control group consists of healthy ovulatory women without any evidence of PCOS, enrolled from members of the hospital staff, and students within the same age range as the test group.

Inclusion and Exclusion criteria:

Only subjects who met the inclusion criteria and gave informed consent were recruited. The control subjects with the regular menstrual cycle, not taking oral contraceptives or breastfeeding were enrolled.

Ethical Consideration:

The subjects were adequately informed about the study objective and only those who met the inclusion criteria and gave written consent were enrolled. The socio-demographic characteristics and relevant gynecological history were obtained using a structured questionnaire. The research protocol was reviewed and approved by the Ethics committee of the Central Hospital, Warri, Delta State (Ref: CHW/ECC Vol 1/104). Absolute confidentiality, subject autonomy, and non-maleficence, amongst others, were strictly followed to protect the study group and control subjects. Their details were coded, no names were documented, such that no data could be traceable to the subjects.

Sample size

The sample size (N) was calculated using the sample size determination formula for health studies (15) and 2.2% prevalence of PCOS among infertile women conducted previously in Nnewi, Nigeria (16).

$$N = \frac{Z^2 P(1-P)}{D^2}$$

N = 33 minimum sample sizes

However, 40 subject samples were recruited for this study due to attrition.

Sample collection

Under strict aseptic precautions, 5mL of venous blood was obtained from each of the participants and transferred into the plain bottle. Samples were centrifuged at the speed of 3000rpm for 5 minutes and sera were separated. The serum recovered was transferred into a fresh tube and stored at -20^{0}c. Serum LH, FSH were determined using the ELISA technique.

Laboratory Analyses

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Serum LH and FSH were assayed by Enzyme-linked Immunosorbent assay technique using reagent supplied by Monobind Inc, Lake Forest, California, United States of America.

The working dilution of washing buffer from the concentrated buffer and the working substrate solutions were prepared according to the manufacturer's protocol prior to commencement of assay. The desired number of microwells from the zip-lock bag were removed and placed in a microwell holder. Then, 50µL of each reference standards, control and test sample were dispensed into the appropriate well and 100µL of enzyme conjugate was dispensed into each well. The wells were then gently rocked for 20 seconds and sealed by covering with parafilm coated foil paper and incubated at room temperature for 60minutes. The cover slip was then removed and discarded and incubation mixture was thoroughly decanted by flicking into a sink containing disinfectant. The microwells were then washed five times with diluted washing Buffer. The wells were dried by firmly tapping the plate on a clean paper towel to remove excess washing solution. Thereafter, 100µL of working substrate reagent was dispensed into each well and incubated at room temperature for 15minutes. The reaction was stopped by adding 50µL of stopping solution to each well and gently rocking the wells. The absorbance of each well read at 450nm in a microplate reader**.**

A standard curve was obtained by plotting the LH or FSH concentrations of reference standards versus the corresponding absorbance. The concentrations of LH and FSH were extrapolated from the standard curves.

Statistical Analysis: Data were analyzed using the statistical software for social sciences (SPSS) version 20.0. The data were expressed in terms of descriptive statistics mean ± standard deviation. Students-t-test was used to compare means between cases and controls while the Pearson correlation coefficient was used to associate the LH/FSH ratio with age and BMI. P-value ≤0.05 was considered as statistically significant.

RESULTS

A total of 80 women were recruited into the study. This comprised of 40 infertile women with PCOS and 40 healthy women of proven fertility as controls. Table 1 shows the socio-demographic parameters of the study population. The mean BMI of the women with PCOS was significantly higher than controls, but the difference in the mean age was not statistically significant. Table 2 describes the comparisons of serum LH and FSH in infertile women with PCOS and control subjects. The mean LH and FSH levels were significantly higher ($p<0.001$) among the infertile women with PCOS when compared to the fertile controls. The LH/FSH ratio was significantly higher ($p<0.001$) among women with PCOS than control subjects. The serum LH and FSH correlated with age ($r=0.318, p<0.05$; $r=0.320$, $p<0.05$) respectively among women with PCOS. There was a significant correlation between LH and BMI but not between FSH and BMI. On the other hand, LH/FSH ratio correlated with both age and BMI ($r=0.319$, $p<0.05$; $r=0.309$, $p<0.05$) among women with PCOS.

Table 1: Demographic characteristics of study participants

Characteristics of subjects	Women with PCOS	Control subjects	P-value
Number of subjects	40	40	-
Age (Years)	32.35±2.00 (21-47yrs)	33.31 ± 0.20 (20-45)	0.08
Body mass index(Kg/m^2)	31.5±0.5	30.2±0.4	0.02
Age of menarche (Years)	12.2±0.1	12.4±0.1	0.6
Level of Education			
Primary Education	05(12.5)	06(15)	0.6
Secondary Education	23(57.5)	26(65)	0.08
Tertiary Education	12(30)	08(20)	0.05
Marital status			
Married	36(90)	39(97.5)	0.9
Single	04(10)	01(2.5)	0.05

Table 2: Comparison of serum LH and FSH in infertile women with PCOS and control subjects.

MEASURED VARIABLES (Reference Range)	WOMEN WITH POLYCYSTIC OVARIAN SYNDRONE (PCOS) n= 40	FERTILE WOMEN (Controls) n=40	P-value
Age (year)	32.35±2.00 (21-47yrs)	33.31 ± 0.20(20-45)	0.08
LH (µIU/ml) (0.5 – 10.5)	20.02±2.00(13.9 – 36.7)	4.56 ± 0.20 (1.5 – 9.0)	0.001
FSH (µIU/ml) (3.0 – 12.0)	8.66±2.00 (1.1 – 24.5)	7.24 ± 0.003 (3.0 – 10.5)	0.001
LH/FSH Ratio	2.32±0.02	0.62±0.01	0.001

***Values in parenthesis is confidence interval**

Table 3: Correlation of LH, FSH and LH/FSH ratio with age and BMI

Parameters	Age		Body mass index	
	R-value	P-value	R-value	P-value
LH	0.318	0.05*	0.320	0.05*
FSH	0.320	0.05*	0.276	0.1
LH/FSH	0.319	0.05*	0.309	0.05*

* indicates significant p value

DISCUSSIONS

This study was conducted to determine the levels of LH, FSH, and LH/FSH ratio among women diagnosed with PCOS and to correlate their values with BMI and age of participants. The Rotterdam consensus criteria did not include LH/FSH ratio as part of the diagnostic criteria for PCOS even though earlier studies have suggested its usefulness (11,12). The suggested use of the LH/FSH ratio in the diagnosis of PCOS is controversial. Whereas some have reported that LH/FSH ratio greater than 2 can be accepted as abnormal in the diagnosis of a subgroup of individuals with PCOS (11), others have expressed concerns about the clinical utility of the ratio (13). The earlier study reported that 45.4%; of subjects with PCOS had elevated LH/FSH ratio while 55% had normal gonadotropin ratio. However, statistically significant differences were observed between groups with normal and elevated LH/FSH ratio when other parameters such as BMI, serum insulin, and LH levels were included (11).

In this study, it was observed that serum LH, FSH levels were significantly higher ($p<0.001$) than controls. The LH/FSH ratio was higher ($p<0.001$) and greater than 2.0 among women with PCOS than controls. This is consistent with previous studies (11,12). The authors observed that LH: FSH ratio is a valuable diagnostic tool in evaluating Nigerian women with PCOS. Other authors elsewhere indicated that not all women with PCOS possess hormonal and biochemical changes suggestive of the disease (14).On the contrary, a study that was conducted to determine the predictive value of the LH/FSH ratio among women with PCOS observed that LH/FSH of subjects with PCOS was 1.25 against controls which were 0.71. The authors concluded that even though LH/FSH ratio is a valuable test, it is not diagnostic of PCOS (17). The reported LH/FSH ratio by these authors was lower than 2.32 reported in the present study. Several conflicting results are available in the literature concerning the association of LH: FSH ratio with BMI. Some studies observed that the none obese PCOS women had a significantly higher level of serum LH than obese counterparts (18) while Yanira et al (19) observed that an inverse relationship between LH and BMI in PCOS, the latter study suggests that the effect of BMI on LH has mediated at the pituitary level and not a hypothalamic level in those patients. Kiddy et al (20) had earlier reported an inverse correlation of FSH with BMI in obese PCOS and there is an increased frequency of hirsutism in obese compared with lean PCOS women. We observed significant correlations between

mean LH/FSH ratio and BMI and age among the study group.

Abnormality of the hypothalamic-pituitary-ovarian or adrenal axis has been implicated in PCOS. Disturbance in the secretion and release of gonadotrophin-releasing hormone (GnRH) results in the relative increase in LH to FSH release (14). An abnormal feedback mechanism by ovarian estrogen is also implicated to play a role in this imbalance LH-FSH release. As a result of this disequilibrium between FSH and LH levels, the ratio may be elevated in about 60% of the women with PCOS (14). These authors reported that a raised LH/FSH ratio greater than 2.0 was observed in 64% of the women with PCOS (14). In a large population study of 745 women who were treated for infertility, PCOS patients were observed to have different ranges of LH: FSH ratios from < 1 range to 4.6–5.5, and subjects were classified into 7 PCO subgroups based on their LH: FSH ratios. Also, BMI, LH, FSH, LH: FSH ratio, insulin, HbA1c, estradiol, testosterone, and TSH were significantly higher ($P < 0.05$) compared to controls (21).

The mean BMI of women with PCOS was significantly higher ($p<0.02$) than control subjects, but both cases and control were mildly obese with a BMI greater than 30kg/m^2. Some authors have suggested that compared to normal-weight women with PCOS, obese women with PCOS have increased prevalence of glucose intolerance and diabetes mellitus type 2 (10), increased prevalence of hirsutism, higher risk of metabolic syndrome and risk of cardiovascular disease and high level of PAI-1(plasminogen activator inhibitor-1) observed in PCOS patients may add to increased cardiovascular risk (22). Obesity increases the prevalence of obstructive sleep apnea in patients with PCOS. Dysregulation of lipolysis in PCOS patients has been reported, because of increased lipolysis of visceral fat with a consequent increase of free fatty acids released directly into the portal circulation. The high lipolysis at the visceral fat may be one of the mechanisms for the increased risk of glucose intolerance (23). In obese women, low-calorie diet, exercise, and the reduction of body fat may lead to an improvement of ovarian function with the restoration of spontaneous ovulation and reduced risk of type 2 diabetes mellitus. It was observed that BMI is potentially associated with the endocrine environment of subjects with PCOS. Some authors stated that with PCOS and higher BMI seemed more likely to suffer hyperandrogenism or to exhibit the clinical signs of androgen excess (24). In such women with PCOS and high BMI, it is necessary to explore the pathogenesis of acne and hirsutism (6). Increased adipose mass is associated with the production of numerous factors including aromatase, leptin, plasminogen activator inhibitor 1, insulin resistance, and dyslipidemia, all of which can lead to tissue damage. Obese patients with PCOS have been shown to exhibit significantly more severe insulin resistance than obese women (24).

Conclusion

The result of the study showed that serum LH and FSH levels as well as LH: FSH ratio were significantly higher among women with PCOS than controls. Accordingly, LH: FSH ratio correlated with age and BMI in women with PCOS. The LH: FSH ratio may be useful as a diagnostic index among this group of subjects. Further study involving a large study population is suggested to ascertain the diagnostic utility of the LH/FSH ratio among Nigeria women with PCOS.

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REFERENCES

1. Rehman R, Mehmood M, Ali R, Shaharyar S, and Alam F. Influence of body mass index and polycystic ovarian syndrome on ICSI/IVF treatment outcomes: A study conducted in Pakistani women, Intern J Reprod Biomed (Yard), 2018;16(8): 529-534.

2. Mckittrick M. Diet and Polycystic ovarian syndrome. Nutr today,2002; 37: 63-69.

3. Moran L, Pasquali R, Teede H, Hoeger KM, Norman RJ. Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and PCOS Society. Fertil Steril, 2009;92: 1966-1982.

4. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Human Reproduction. 2004;19:41–47. https://doi.org/10.1093/humrep/deh098

5. De Leo V, La Marca A. Petraglia F Insulin-lowering agents in the management of polycystic ovary syndrome. Endocrinol Rev, 2003;24:633–667.https://doi.org/10.1210/er.2002-0015

6. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal, and metabolic aspects of PCOS: an update. Reprod Biol Endocrinol, 2016; 14:38.

7. Escobar-Morreale HF, Luque-Ramírez M, San Millán JL. The moleculargenetic basis of functional hyperandrogenism and the polycystic ovary syndrome. Endocr Rev. 2005;26:251–82.

8. Ozgun MT, Uludag S, Oner G, Batukan C, Aygen EM, Sahin Y. The influence of obesity on ICSI outcomes in women with polycystic ovary syndrome. J Obstet Gynaecol, 2011;31:245–249.

9. Huang K, Liao X, Dong X, Zhang H. Effect of overweight/obesity on IVF-ET outcomes in Chinese patients with polycystic ovary syndrome. Intern J Clin Experiment Med. 2014;7:5872–5876.

10. Pasquali R, Gambineri A, Pagotto U. The impact of obesity on reproduction in women with polycystic ovary syndrome. Brit J Obstet Gynaecol, 2006;113:1148–1159.

11. Banaszewska B, Spaczynski RZ, Pelesz M, Pawelczyk L. Incidence of Elevated LH/FSH Ratio in Polycystic Ovary Syndrome Women with Normo- And Hyperinsulinemia, Rocz Akad Med Bialymst; 2003;48:131-134.

12. Akpata C, Uadia P, Okonofua F. Prevalence of Polycystic Ovary Syndrome in Nigerian Women with Infertility: A Prospective Study of

the Three Assessment Criteria. Open J Obstet Gynecol, 2018; 8:1109-1120. doi: 10.4236/ojog.2018.812112.

13. Choi LW, Jayagopal V, Kilpatrick VE, Holding S, Atkin SL. The LH/FSH ratio has little use in diagnosing polycystic ovarian syndrome. Ann Clin Biochem, 2006;; 43: 217–219.

14. Al-Nakash AH and Al-Taee NK. Polycystic Ovarian Syndrome: The Correlation between LH/FSH Ratio and Disease Manifestations. Mid East Fertil Soc J, 2007; 12, 35-40.

15. Lwange SK, Lemeshow S. Sample size determination in health studies; a practical manual. IRIS, World Health Organization, 1991.

16. Igwegbe AO, Eleje GU, Enechukwu CI. Polycystic ovary syndrome: a review of management outcomes in a low resource setting. J Women's Health Issues Care,2013; 2:3.[http://dx.doi.org/10.4172/2325-9795.1000110].

17. Saucedo de la Llata E, Moraga-Sánchez MR, RomeuSarrió A, Carmona-Ruiz IO. Proporción LH-FSH y síndrome de ovario poliquístico: prueba olvidada o no útil Ginecol Obstet Mex 2016;84(2):84-94.

18. Insler V, Shoham Z, and Barash A. Polycystic ovaries in none obese and obese patients: a possible pathophysiological mechanism based on a new interpretation of facts and findings. Hum Reprod, 1993; 8(3):379-84.

19. Yanira LP, Serene SS, Yarisie J, Anne E, Sabrina G, and Janet EH. Inverse Relationship between Luteinizing Hormone and Body Mass Index in Polycystic Ovarian Syndrome. Investigation of Hypothalamic and Pituitary Contributions. J Clin Endocrinol Metab 2006;91(4):1309-1316.

20. Kiddy DS, Sharp PS, and White DM. The difference in clinical and endocrine features between obese and nonobese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases. Clin Endocrinol Oxf. 1990 32(2):213-20.

21. Malini NA and George KR. Evaluation of different ranges of LH: FSH ratios in Polycystic Ovarian Syndrome-Clinical Based Case-control study. Gen Comp Endocrinol,2018; 260:51-57.

22. Desai V, Prasad NR, Manohar SM, Sachan A, Narasimha SR, Bitla AR. Oxidative stress in non-obese women with the polycystic ovarian syndrome. J Clin Diagn Res 2014;8:CC01-03.

23. Hussein B, Alalaf S. Prevalence and characteristics of a polycystic ovarian syndrome in a sample of infertile Kurdish women attending IVF infertility center in the maternity teaching hospital of Erbil City. Open J Obstet Gynecol 2013;3:577-85.

24. Yuan C, Liu X, Mao Y, Diao F, Cui Y, and Liu J. Polycystic ovary syndrome patients with high BMI tend to have functional disorders of

androgen excess: a prospective study. J Biomed Res, 2016; 30(3): 197–202.