Original Research

The Study of the AMH, SHBG, Free Androgen Index and LH/ FSH Ratio in the Diagnosis of Polycystic Ovary Syndrome in Adolescent

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) diagnosis is controversial in adolescents. Therefore, auxiliary markers are required for the diagnosis of PCOS.

Aim and Objective: We aimed to evaluate whether luteinizing hormone (LH)/ follicle-stimulating hormone (FSH) ratio, free androgen index (FAI), anti-Mullerian hormone (AMH), and sex hormone-binding globulin (SHBG) levels are a useful test to screen adolescents with PCOS and to investigate which of them has more diagnostic value in the PCOS diagnosis.

Material and Methods: A total of 28 girls with PCOS and 35 healthy girls consisted in this study. Pediatric Endocrine Society criteria were used to diagnose PCOS. Clinical examinations and hormonal assays were performed.

Results: The LH/FSH ratio, and FAI levels were detected significantly higher, and SHBG levels were detected significantly lower in the PCOS group than in the control group ($p<0.001$). The best marker for PCOS diagnosis was found as AMH. In all adolescents with PCOS, irrespective of obesity/overweight, significantly higher AMH levels were observed compared to the control subjects ($p<0.001$). Also, we measured a LH/FSH ratio cut-off value of 1.48 ng/ml with 77% sensitivity and 77% specificity to differentiate cases with PCOS from healthy controls.

Conclusion: AMH, FAI, and LH/FSH ratio could be useful and valuable tests for the PCOS diagnosis in the presence of the PCOS criteria. AMH was found to be the strongest diagnostic marker in patients with PCOS.

Key Words: AMH, FAI, LH/FSH ratio, PCOS, SHBG

INTRODUCTION

Polycystic ovary syndrome (PCOS), which is a current problem of reproductive age, affects 3.6-15% of women [1,2]. Generally, Rotterdam consensus criteria are used for diagnosis. Rotterdam consensus criteria include a combination of anovulation, polycystic ovary, and hyperandrogenism (HA) in adults [3]. Since ovarian physiology in adolescents is slightly different from that of adult women, different consensus criteria have been established to avoid underdiagnosis and overdiagnosis in adolescents [4]. The menstrual cycles of adolescents differ from those of adults; therefore, anovulation criteria should be appropriate for the age and pubertal stage [5]. Physiological anovulation in adolescents should not be confused with PCOS. Therefore, it is important whether menstrual irregularities continue or not. Clinical or biochemical HA is the diagnostic criterion for PCOS. Acne, hirsutism, alopecia, and menstrual irregularity are the findings of hyperandrogenism. Since acne and mild hirsutism are normal signs of puberty, mild hirsutism alone and isolated acne does not suggest hyperandrogenism [6]. It has been reported that if mild hirsutism is detected in the presence of menstrual irregularity, this may be a marker of

androgen excess [7]. Polycystic ovary syndrome (PCOS) also known as Stein Leventhal syndrome is one of the most common endocrine abnormalities among premenopausal women, yet its diagnosis remains one of the most challenging issues in endocrinology and reproductive medicine [8]. It has been recognized as the most frequent endocrinopathy among reproductive-aged women, with a prevalence of 6- 10% world-wide [9]. There is a recent rise in PCOS cases in urban India because of westernization, modernization, stress and life style changes.Modified Ferriman Gallwey (mFG) score was used for the evaluation of hirsutism [10]. In addition, the free androgen index (FAI) is one of the methods used for the evaluation of hyperandrogenism, but studies on this subject in adolescents are rare [11]. Polycystic ovarian morphology (PCOM) is not accepted as a criterion for PCOS, as PCOM is a normal finding in many healthy adolescents [12].The pathophysiology of PCOS is still not fully understood and it has been shown that disorders of the adrenal or hypothalamuspituitary-ovarian axis have a major role in this topic. Secretion defects in gonadotropin-releasing hormone (GnRH) cause a relative increase in luteinizing hormone (LH) secretion [13]. Studies demonstrated that the LH/ follicle-stimulating hormone (FSH) ratio increase in women with PCOS [14]. Also, studies have suggested that serum anti-Mullerian hormone (AMH) level has increased significantly in women with PCOS compared to healthy women [15,16]. Human sex hormone binding globulin (SHBG), which binds androgens and estrogens with high affinity and specificity, is produced in the liver [17]. It was demonstrated that binding and transporting sex steroids affect the bioavailability of these hormones (16). Meta-analysis showed that metabolic abnormalities in women with PCOS were associated with obesity, which was associated with low SHBG levels, and not with hyperandrogenism indices. This highlights the possibility that before increasing androgen levels in PCOS, decreasing SHBG levels occur [18]. The current study aimed to evaluate LH/FSH ratio, FAI, AMH, and SHBG levels to represent a useful and practical test to screen adolescents for PCOS and to investigate which of them has more diagnostic value in the diagnosis of PCOS.

MATERIALS AND METHODS

Adolescents diagnosed with PCOS and healthy control group between May 2022 and May 2023 were included in this prospective study. The diagnosis of PCOS was made when two features of the syndrome were present: an abnormal uterine bleeding pattern consisting of oligo-amenorrhea or excessive uterine bleeding and clinical and/or biochemical signs of HA. Secondary amenorrhea was defined as follows: > 90 days without a menstrual period after initial menstruation; oligomenorrhea was defined as; 2nd year of menarche: average cycle length > 60 days; 3rd year of menarche: average cycle length > 45 days; 4th year of menarche: cycle length > 38 days. In the presence of a menstrual cycle with intervals of less than 21 days, or when menstruation lasts longer than 7 days, or having heavy menstruation (more than one pad needs to be changed every 1-2 hours, or clots) were defined as excessive uterine bleeding [12,20]. HA can be classified as clinically and biochemically. The presence of a mFG score ≥8 and/or moderately severe inflammatory acne vulgaris was evaluated as clinical HA. A score of 8 - 15 indicates mild hirsutism and >15 indicates moderate or severe hirsutism. In premenopausal Caucasians, mFG score >8 is considered above the 95th percentile for the population in adult women (5). Testosterone measured above adult norms was evaluated as biochemical HA. Over 50 ng/dL was accepted as high according to our laboratory. The additional inclusion criteria were: menstruation for at least 2 years after first menstruation, persistent symptoms for 1–2 years, absence of other endocrine diseases, inherited syndromes and congenital malformations, and not using drugs (including oral combined contraceptives) for 3 months before the study. The control group subjects were healthy adolescent girls without gynecological or endocrine pathology. The healthy patient group was selected from patients who were referred to endocrinology with complaints of menstrual irregularity in the history, increased hair growth, and cysts on ultrasonography, but whose menstrual cycle was found to be normal according to their gynecological age, who did not continue to have menstrual irregularities in their followup, and who did not have hyperandrogenism in the endocrine evaluation. This study was approved by NCMCH Ethics Committee .

LABORATORY AND CLINICAL MEASUREMENTS

Anthropometric evaluations (body weight, height, body mass index (BMI)) were done by the same physician. The BMI was assessed using the ratio of weight (kg) to height squared (m2). Assessment of hirsutism was graded according to the mFG score by the same physician. After an overnight fast, fasting blood samples for glucose, insulin, LH, FSH, total testosterone, estradiol, progesterone, SHBG, and AMH were drawn between 08:00-09:00 a.m in the follicular phase 1-7 days after spontaneous menstruation for controls and at a convenient time for PCOS group. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated (HOMA-IR = fasting plasma insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5). The FAI was calculated with the following formula: total testosterone x 100/SHBG. The plasma glucose levels were measured by an enzymatic colorimetric method. Insulin, FSH, LH, testosterone, and estradiol levels

were measured using a chemiluminescence immunoassay and AMH levels were measured by an enzyme-linked immunosorbent assay by VIDAS AMH assay by bioMérieux. AMH values of > 9 were not measured in our hospital ELISA assay kit. This was defined as a score >9 in the laboratory. So, data of the patients with an AMH value >9 were entered as 9 in our study

STATISTICAL ANALYSIS

Statistical analyses and evaluations were performed with SPSS 16.0. The mean, standard deviation (SD), median, and 1st (Q1) and 3rd (Q3) quartiles of the numerical variables were calculated. Categorical variables are expressed as numbers and percentages (%). The Shapiro-Wilk test was used to evaluate the normal distribution of variables. Furthermore, the variables with kurtosis and skewness values in the range of -1.5 to 1.5 were considered to have a normal distribution. Student's T-test was performed for groups with normal distribution, and the Mann-Whitney U test was performed for groups that did not comply with the assumption of normal distribution. Chi-square tests were performed for comparing categorical variables. The PCOS and control groups were divided into four groups according to whether they were overweight/obese or normal. Oneway analysis of variance (ANOVA) was used to evaluate the statistical differences between groups with normal distribution, and Kruskal Wallis test was used to evaluate those who did not. Intra-group differences in AMH levels were evaluated using post-hoc analysis. Tamhane's T2 test was used for the analysis. Intra-group differences in LH/FSH ratios were evaluated using post-hoc analysis. Tukey test was used for the analysis. In addition, the laboratory markers used to predict the presence of polycystic ovary syndrome were analyzed using binary logistic regression analysis. Logistic regression model was used to identify predictors of the dependent variable, if more than one independent predictor variable was evaluated, p value <0.250 in univariate analysis, tested using multivariate logistic regression analysis of clinically significant variables. The LH/FSH ratio, FAI, SHBG, and AMH levels were evaluated with logistic regression analysis to predict the presence of polycystic ovary syndrome. For LH/FSH ratio, the best cut-off value that could be used to differentiate between children with PCOS and healthy controls was calculated using receiver operating characteristic (ROC) curve analysis. The p value for statistical significance was set at $p < 0.050$.

RESULTS

A total of 63 adolescents, 28 (44.44%) patients diagnosed with PCOS, and 35 (55.56%) healthy controls were included in our study. All pubertal patients were Tanner stage. In terms of mFG score, the cases were divided into subgroups according to clinical evaluation as mFG score <8, mFG score 8-15, and mFG score >15. In the healthy control group, the score of 69 (98.6%) cases was lower than 8, and the score of one case was 8-15. In the PCOS group; the score of 23 (80.4%) cases was 8-15, and the score of 6 (19.6%) cases was higher than 8. Both PCOS and the healthy control group were similar age (p=0.429). The clinical and laboratory characteristics of the polycystic ovary syndrome group and healthy controls are presented in Table NO.1. The LH, LH/FSH ratio, total testosterone, insulin, HOMA-IR, FAI, and AMH levels were measured significantly higher in the PCOS group (p<.001). While the AMH value was >9 in 4 patients in the PCOS group, the AMH value was >9 in 1 patient in the healthy control group. SHBG levels were found to be significantly lower in the PCOS group compared to the control group (p<0.001). The effect of LH/FSH ratio, FAI, SHBG, and AMH levels on the likelihood that cases have PCOS was determined by logistic regression analysis. The logistic regression model was statistically significant ($\chi2$ (5) = 122.597, p<0.001). The model explained 87% (Nagelkerke R2) of the variance in PCOS and correctly classified 95% of the cases. Of all cases predicted to have polycystic ovary syndrome, 91.8% were correctly predicted (The positive predictive value). Of all cases predictedto not have PCOS, 97.1% were correctly predicted (The negative predictive value). Increased FAI and AMH levels were associated with an increased likelihood of PCOS. The univariate binary logistic regression analysis results for factors that predict PCOS are shown in Table II. We regrouped adolescents with PCOS and controls according to BMI as overweight/obese PCOS patients, normal-weight PCOS patients, overweight/obese controls, and normal-weight controls and again compared all variables (Table III). The BMI SDS measurement and FAI levels were detected significantly higher in the PCOS group with overweight/obese compared to the other 3 groups, and SHBG levels were found to be lower. In all adolescents with PCOS, irrespective of obesity/overweight, significantly higher AMH levels were found compared to the healthy control subjects (p<0.001). Among the children with PCOS, those who had normal-weight had higher AMH levels than those who were obese or overweight (p=0.005). Among healthy children, there was no statistically significant difference between the AMH levels of those who had normal-weight and those who were obese or overweight (p=0.472). We detected an LH/FSH ratio cut-off value of 1.48 ng/ml with 77% sensitivity and 77% specificity, a 77% positive predictive value, and a 77% negative predictive value to differentiate cases with PCOS from healthy controls (Figure 1). The maximum area under the curve (AUC) for the mean LH/FSH ratio was 0.81 (95% CI:0.73-0.88; p<0.001).

Table No. 1: The clinical and laboratory characteristics of polycystic ovary syndrome group and healthy controls

	Polycysticovary syndrome (n=28)		Healthy controls (n=35)		P-value
	Mean±SD	Median Q1–Q3	Mean±SD	Median Q1–Q3	
Age (years)	16.1±1.3	16 (15.1–17.4)	15.9±1.3	16.1 (14.8–17)	0.429
Weight (kg)	76.7±18.5	74.6 (61.5–91.3)	59.1±11.8	56.4 (50–70)	<0.001
Height (cm)	163.2±6.3	162.9 (159.3–168)	161.1±5.8	162 (156.1–165)	0.057
BMI (kg/m2	28.6±6	28.5 (23.3–33.8)	22.7±4	22 (20.1–24.9)	<0.001
FSH (mIU/mL)	6.6±1.7	6.5 (5.2-7.9)	5.7±1.7	5.7 (4.7–6.7)	0.004
LH (mIU/mL)	13.8±7.3	12.5 (8.1–19.8)	6.1±5.6	4.6 (2.9–7.4)	<0.001
LH/FSH ratio	2.08±1.05	2 (1.5–2.6)	1.09±0.83	0.81 (0.52–1.44)	<0.001
Estradiol (pg/mL)	55.1±24.7	49.5 (39.366)	83±88.5	48(34–102.3)	0.768M
Testosterone (ng/dL)	49.3±20.8	49 (35–56)	25.9±8.5	24 (1931.5)	<0.001M
Progesterone (ng/mL)	0.98±1.1	0.74 (0.51–1.1)	1.89±3.1	0.73 (0.4–1.47)	0.854M
SHBG (nmol/L)	24.4±14.1	20.5 (13–32.8)	47.3±19	45 (33–56.3)	<0.001M
FPG (mg/dL)	86.6±7.5	87 (82.3–90)	84.9±6.3	85 (80–89)	0.179M
Insulin (ng/mL)	21±13.1	17.7 (11.2–25.3)	11±5.4	9.4 (7.7–12.3)	<0.001M
HOMA-IR	4.56±3.02	3.64 (2.38–5.75)	2.35±1.27	1.98 (1.68–2.63)	<0.001M
FAI	2.81±2.16	2.15 (1.2–3.77)	0.63±0.3	0.51 (0.4–0.76)	<0.001M
AMH (µg/l)	6.85±2.19	7.65 (5.18–9)	3.47±1.82	3 (2.08–4.4)	<0.001

Table No. 2: Univariate binary logistic regression analysis results of factors that predict polycystic ovary syndrome presence

Predicting factors	*B*-value	Odds ratio	%95 Confidence interval		P-value
LH/FSH ratio	0.937	2.553	1.079	6.043	0.033
FAI	4.386	80.305	5.034	1281.044	0.002
SHBG	-0.017	0.983	0.917	1.054	0.637
AMH	0.716	2.046	1.373	3.050	0.001

Table No. 3: Differences in PCOS and control groups according to being overweight and obese, and not.

	Overweight/obese PCOS* (n=18)	Normal weight PCOS* (n=10)	Overweight/obese controls* (n=13)	Normal weight controls* (n=22)	p -value
Age (years)	16.2±1.4	16±1.3	15.9±1.3	16±1.4	0.842
Weight (kg)	87±14.5	59.6±9.3	71.6±7	51.2±5.8	<0.001
Height (cm)	163.3±6	163.1±7	163.9±5.5	159.4±5.3	0.004
BMI (kg/m2)	32.3±3.9	22.3±2.6	26.7±2.9	20.2±2	<0.001
Basal LH	10.7±5.5	18.8±7.2	5±5.2	6.8±5.8	<0.001
LH/FSH ratio	1.76±0.76	2.62±1.25	0.92±0.7	1.19±0.89	<0.001†
SHBG	20.8±12.7	30.5±14.4	42.3±15.9	503±20.2	<0.001
FAI	3.26±2.3	2.09±1.73	0.71±0.33	0.57±0.28	<0.001†
AMH	6.14±2.3	8.02±1.41	2.95±1.64	3.78±1.88	<0.001

Among the children with PCOS, those who had normal-weight had higher LH/FSH ratios than those who were obese or overweight (p=0.003). Among healthy children, there was no statistically significant difference between the LH/FSH ratios of those who had normal-weight and those who were obese or overweight (p=0.616).

DISCUSSION

We studied several biochemical factors that produced varying findings in the diagnosis of PCOS.

We report increased FAI, LH/FSH ratio, AMH, and lower SHBG levels in adolescents with PCOS compared to controls. The most accurate sign for PCOS diagnosis has been found by the AMH. Compared to the other three groups, overweight/obese adolescents with PCOS had lower SHBG levels and higher FAI. Adolescents with PCOS had considerably greater AMH levels than healthy controls, regardless of BMI. Furthermore, we discovered that the AMH levels of the normal-weight PCOS group were greater than those of the obese/overweight PCOS group. It's interesting to note that compared to the obese/overweight PCOS group, the normal-weight PCOS group had a greater LH/FSH ratio. As previously reviewed, PCOS diagnosis are controversial and may lead to misdiagnosis in adolescents. So, studies have been conducted to identify newer biomarkers to aid in diagnosis.The potential utility of AMH as a PCOS diagnostic or supplementary criterion has been evaluated. Serum AMH testing is valuable for the diagnosis of PCOS in women, as demonstrated by Sahmay et al. When comparing women with PCOS to healthy controls, they found that their serum levels of AMH were greater [21]. Similar findings were suggested by another Chinese study. According to reports, PCOS can be accurately diagnosed and its specificity and sensitivity can be increased by combining markers such serum testosterone, serum AMH, LH/FSH ratio, and fasting insulin [22].Moreover, it was reported that AMH levels were higher in non-obese and obese adolescents with PCOS compared to the control group. It has been shown that AMH levels can decrease with weight loss or other treatments in adolescents diagnosed with PCOS [23,24]. Also some studies demonstrated a significant negative relationship between BMI and AMH, some studies suggested that AMH was not statistically different for obesity but rather correlates with PCOS status [25-26].The correct threshold is unknown, despite the fact that several AMH cutoff values with varied sensitivities and specificities have been proposed up to this point. The AMH threshold value of 4.7 ng/mL was found to have specificity and sensitivity of 79.4% and 82.8%, respectively, in women with PCOS, according to a meta-analysis [28]. AMH level > 7.20 ng/mL had the highest sensitivity (76.0%) and specificity (89.0%) for PCOS diagnoses in adolescence, according to another investigation on adolescent PCOS [29]. Our study was unable to measure AMH levels more than 9, so it was unable to determine the AMH cut-off value. One of the present study's shortcomings is this. Serum AMH levels were considerably higher in PCOS patients than in healthy controls, which is consistent with the literature, even if AMH values >9 were considered the lowest value, such as 9. In addition, our study showed that serum AMH levels were considerably higher in PCOS-affected adolescents, irrespective of BMI. According to our studies, patients with PCOS who are normal weight had greater AMH levels than those who are obese or overweight. But in the healthy control group, there was no difference in the AMH levels of obese and normal-weight teenagers.Even though high serum AMH levels are associated with PCOS, studies vary in their findings due to the use of various AMH test methods and PCOS criteria. AMH level overlap was found to be considerable in addition to study heterogeneity [30]. The diagnosis of PCOS and the identification of polycystic ovary shape should not be made solely primarily on the AMH value, according to evidence-based guidelines from a systematic review [28]. When the above-mentioned PCOS characteristics are present, the combination of the AMH and LH/FSH ratio may be a practical and helpful criterion for PCOS diagnosis. According to Khashchenko et al. (29) the diagnosis of PCOS was very sensitive and specific when the LH/FSH ratio was greater than 1.23. The LH/FSH ratio cut-off was shown to have a greater specificity but a lower sensitivity of 1.33 in another investigation. 95.24% and 65.76%, in that order. In this investigation, it was also discovered that the LH/FSH ratio and serum AMH level were equally useful in distinguishing PCOS patients from controls [14]. There was no difference between the two groups in a different study assessing the LH/FSH ratio in PCOS patients based on BMI (normal-high) levels [31]. It was demonstrated that LH, SHBG, and AMH levels were considerably lower in obese and overweight women compared to normal weight women with various PCOS phenotypes and obese and normal weight healthy control participants and overweight women compared to normal weight women in all groups [25].In our study, similar to the literature, LH, AMH levels were found to be lower in the obese group with PCOS than in the normal weight group with PCOS, but no difference was found between the two groups in the healthy control group. In this case, it made us think that AMH, LH might be related to PCOS condition rather than BMI value.There is little evidence linking SHBG levels to PCOS. PCOS risk is increased by obesity, a growing issue among teenagers. Obesity reduces SHBG production and secretion, which is hypothesized to cause PCOS by making androgens more bioavailable [32]. A meta-analysis comparing SHBG and PCOS showed considerable variation between studies, with controls having substantially higher levels of SHBG than PCOS patients. In women with PCOS, these meta-analyses revealed a substantial correlation between low SHBG and obesity, insulin resistance, glucose intolerance, hyperandrogenism, and type 2 diabetes [33]. In another study, in which two groups with similar BMI SDS were evaluated, low levels of SHBG and high levels of AMH were reported in the PCOS group compared to the healthy control group [34]. Moreover, another study suggested that the combination of SHBG and AMH

had higher sensitivity to diagnose PCOS when compared with AMH levels alone [35]. Therefore, SHBG may be a beneficial biomarker to be used in the diagnosis and post-treatmentfollow-up of PCOS. In this current study, SHBG levels in the PCOS group were detected significantly lower than in the healthy control group. We also showed a negative association between SHBG levels and obesity. The serum SHBG levels decrease in individuals with obesity, and there are many studies in this direction in the literature. Therefore, we thought that it would not be appropriate to conduct a cut-off for SHBG directly and indirectly for FAI values. The majority of the data in the literature showed that adolescents with PCOS had greater levels of testosterone, LH, LH/FSH, and FAI than controls [14,36]. Which one is better than other endocrine factors for PCOS diagnosis is the crucial question. The AMH, testosterone, FAI, androstenedione, LH/FSH ratio, ovarian volume, and ovarian-to-uterine index were assessed by Khashchenko et al. [29] in order to predict PCOS. This study showed that the highest accuracy of over 90% was achieved when diagnosing PCOS using four or more of the given criteria. Additionally, they demonstrated that the diagnostic precision was 85% when only three parameters were employed, and that the rate of accurate diagnosis dropped as the number of parameters utilized decreased [36, 37]. The logistic regression model demonstrated that 95% of PCOS cases could be identified with 87% sensitivity using four parameters (the LH/FSH ratio, FAI, AMH, and SHBG). The LH/FSH ratio and AMH were the two model parameters that were significant. In subgroup analyses, these factors varied significantly, particularly in the differential diagnosis between individuals who were obese and those who were not.The strength of this study lies in the homogeneity of our study population and the precise and well-defined delineation of the patient and control groups. The identical doctor assessed each individual. Regarding the study's limitations, we were unable to determine the AMH value larger than 9. Thus, it was not possible to compute the AMH cutoff value. Furthermore, the PCOS and control groups had comparatively fewer participants.

CONCLUSION

The findings of this study imply that serum elevated levels of FAI, AMH, and LH/FSH ratio may be practical and useful assays for identifying teenage PCOS. AMH was determined to be the most effective marker for PCOS diagnosis among them. Furthermore, elevated obesity in PCOS-affected teenagers is associated with a negative correlation with LH/FSH ratio and AMH levels, but not in the healthy group.

Larger research enable us to learn more and come to more accurate conclusions.

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