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

Adölesanlarda Polikistik Over Sendromu Tanısında AMH, SHBG, Serbest Androjen İndeksi ve LH/FSH Oranının Rolü

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ABSTRACT

Objective: Polycystic ovary syndrome (PCOS) diagnosis is controversial in adolescents. Therefore, auxiliary markers are required for the diagnosis of PCOS. 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 56 girls with PCOS and 70 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 usefull 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: Anti-Mullerian hormone, Free androgen index, LH/FSH ratio, Polycystic ovary syndrome, Sex hormone-binding globulin

ÖZ

Amaç: Adölesanlarda polikistik over sendromu (PKOS) tanısı tartışmalıdır. Bu nedenle PKOS tanısı için yardımcı belirteçlere ihtiyaç vardır. PKOS'lu adölesanları taramak ve PKOS tanısında hangisinin tanısal değerinin daha fazla olduğunu araştırmak için Luteinizan hormon (LH)/folikül uyarıcı hormon (FSH) oranı, serbest androjen indeksi (SAI), anti-

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Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. This study was approved by the Ankara Bilkent City Hospital Ethics Committee with the decision no 23-3467 dated March 1, 2023.

Contribution of the Authors / Yazarların katkısı*: *BÜYÜKYILMAZ G: Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***KOCA SB:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***TOKSOY ADIGÜZEL K:*** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in necessary literature review for the study, Reviewing the article before submission scientifically besides spelling and grammar. ***BOYRAZ M:*** Taking responsibility in the writing of the whole or important parts of the study. ***GÜRBÜZ M:*** Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar.

How to cite / *Atıf yazım şekli*: Büyükyılmaz G, Koca SB, Toksoy Adıgüzel K, Boyraz M and Gürbüz M. The Role of the AMH, SHBG, FAI and LH/FSH Ratio in the Diagnosis of Polycystic Ovary Syndrome in Adolescent. Turkish J Pediatr Dis 2024;18:34-40.

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Received / Geliş tarihi : 22.08.2023
Accepted / Kabul tarihi : 27.09.2023
Online published : 06.11.2023
Elektronik yayın tarihi
DOI: 10.12956/tchd.1347807

müllerian hormon (AMH) ve seks hormon bağlayıcı globulin (SHBG) düzeylerinin yararlı ve değerli bir test olup olmadığını değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Bu çalışmaya PKOS tanılı 56 ve sağlıklı 70 kız dahil edildi. PKOS'u teşhis etmek için Pediatrik Endokrin Derneği kriterleri kullanıldı. Klinik muayeneleri ve hormon tahlilleri yapıldı.

Bulgular: PKOS grubunda kontrol grubuna göre LH/FSH oranı ve SAI düzeyleri anlamlı olarak yüksek, SHBG düzeyleri anlamlı olarak düşük saptandı (p<0.001). PKOS tanısı için en iyi belirteç AMH olarak bulundu. PKOS'lu tüm adölesanlarda, obezite/fazla kilodan bağımsız olarak, kontrol grubu ile karşılaştırıldığında anlamlı olarak daha yüksek AMH seviyeleri gözlendi (p<0.001). PKOS'lu vakaları sağlıklı kontrollerden ayırt etmek için LH/FSH oranı eşik değeri %77 duyarlılık ve %77 özgüllük ile 1.48 ng/ml ölçüldü.

Sonuç: AMH, SAI ve LH/FSH oranı, PKOS kriterlerinin varlığında PKOS tanısında yararlı ve değerli testler olabilir. AMH PKOS'lu hastalarda en güçlü tanısal belirteç olarak bulundu.

Anahtar Sözcükler: Anti müllerian hormon, Seks hormon bağlayıcı globülin, LH/FSH oranı, Polikistik over sendromu, Serbest androjen indeksi

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). Modified Ferriman Gallwey (mFG) score was used for the evaluation of hirsutism (8). 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 (9). Polycystic ovarian morphology (PCOM) is not accepted as a criterion for PCOS, as PCOM is a normal finding in many healthy adolescents (10).

PCOS diagnostic criteria in adolescents were revised by the international pediatric subspecialty societies in the 2015 consensus by modifying the "National Institutes of Health criteria" according to age and stage (7). Therefore, PCOS in adolescents typically manifests with a combination of abnormal uterine bleeding patterns and evidence of hyperandrogenism (2,4,10). The difficulty of diagnosing PCOS in adolescents has encouraged studies to search for new markers. Therefore, it is important to understand the pathogenesis of PCOS.

The pathophysiology of PCOS is still not fully understood and it has been shown that disorders of the adrenal or hypothalamus-pituitary-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 (11). Studies demonstrated that the LH/ follicle-stimulating hormone (FSH) ratio increase in women with PCOS (12). Also, studies have suggested that serum anti-Mullerian hormone (AMH) level has increased significantly in women with PCOS compared to healthy women (13,14).

Human sex hormone binding globulin (SHBG), which binds androgens and estrogens with high affinity and specificity, is produced in the liver (15). 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 (17).

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 January 2020 and January 2023 were included in this retrospective 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 (10,18). 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 follow-up, and who did not have hyperandrogenism in the endocrine evaluation. This study was approved by the Ankara Bilkent City Hospital Ethics Committee with the decision no 23-3467 dated March 1, 2023.

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 (Siemens Healthineers, Erlangen, Germany). The SHBG values were analyzed using an IMMULITE 2000 XPi (Siemens, Cary, NC, USA), and AMH levels were measured by an enzyme-linked immunosorbent assay by VIDAS AMH assay by bioMérieux (bioMérieux, Marcy L'Etoile, France). 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 (version 24.0; IBM Corporation, Armonk, NY, USA). 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. One-way 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 126 adolescents, 56 (44.4%) patients diagnosed with PCOS, and 70 (55.6%) healthy controls were included in our study. All pubertal patients were Tanner stage 5. 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 45 (80.4%) cases was 8-15, and the score of 11 (19.6%) cases was higher than 15.

Both PCOS and the healthy control group were similar age (p=0.429). The BMI standard deviation score (SDS) was significantly greater in the PCOS group than in the healthy

Table I: The clinical and laboratory characteristics of polycystic ovary syndrome group and healthy controls

	Polycystic ovary syndrome (n=56)		Healthy controls (n=70)		p
	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
Height SDS	0.18±1.07	0.16 (-0.55–1.09)	-0.12±1.06	-0.06 (-1–0.74)	0.129
BMI (kg/m^2)	28.6±6	28.5 (23.3–33.8)	22.7±4	22 (20.1–24.9)	<0.001
BMI SDS	1.9±1.47	2.2 (0.74–3.1)	0.35±1.45	0.3 (-0.59–1.34)	<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 (39.3–66)	83±88.5	48 (34–102.3)	0.768^M
Testosterone (ng/dL)	49.3±20.8	49 (35–56)	25.9±8.5	24 (19–31.5)	<0.001^M
Progesterone (ng/mL)	0.98±1.1	0.74 (0.51–1.1)	1.89±3.1	0.73 (0.4–1.47)	0.854^M
SHBG (nmol/L)	24.4±14.1	20.5 (13–32.8)	47.3±19	45 (33–56.3)	<0.001^M
FPG (mg/dL)	86.6±7.5	87 (82.3–90)	84.9±6.3	85 (80–89)	0.179^M
Insulin (ng/mL)	21±13.1	17.7 (11.2–25.3)	11±5.4	9.4 (7.7–12.3)	<0.001^M
HOMA-IR	4.56±3.02	3.64 (2.38–5.75)	2.35±1.27	1.98 (1.68–2.63)	<0.001^M
FAI	2.81±2.16	2.15 (1.2–3.77)	0.63±0.3	0.51 (0.4–0.76)	<0.001^M
AMH (µg/l)	6.85±2.19	7.65 (5.18–9)	3.47±1.82	3 (2.08–4.4)	<0.001

Normally distributed variables were evaluated with Student's T test. ***M*** *symbol indicates that Mann Whitney U test was used.* ***SD:*** *standard deviation,* ***SDS:*** *standard deviation score,* ***BMI:*** *body mass index,* ***SHBG:*** *Sex hormone binding globulin,* ***FPG:*** *fasting plasma glucose,* ***HOMA-IR:*** *homeostasis model assessment of insulin resistance,* ***FAI:*** *free androgen index,* ***AMH:*** *anti-Müllerian hormone,* ***LH:*** *luteinizing hormone,* ***FSH:*** *follicle-stimulating hormone.*

Table II: Univariate binary logistic regression analysis results of factors that predict polycystic ovary syndrome presence

Predicting factors	*B*-value	Odds ratio	%95 Confidence interval		p
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

FAI: *free androgen index,* ***SHBG:*** *Sex hormone binding globulin,* ***AMH:*** *Anti-Mullerian Hormone,* ***LH:*** *luteinizing hormone,* ***FSH:*** *follicle-stimulating hormone*

control group. The clinical and laboratory characteristics of the polycystic ovary syndrome group and healthy controls are presented in Table I. 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 15 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 ($\chi^2(5) = 122.597$, p<0.001). The model explained 87% (Nagelkerke R^2) 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 predicted to 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

Table III: Differences in PCOS and control groups according to being overweight and obese, and not.

	Overweight/obese PCOS* (n=35)	Normal weight PCOS* (n=21)	Overweight/obese controls* (n=26)	Normal weight controls* (n=44)	p
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
Height SDS	0.2±1.03	0.16±1.17	0.44±1.05	-0.48±0.91	0.002
BMI (kg/m^2)	32.3±3.9	22.3±2.6	26.7±2.9	20.2±2	<0.001
BMI SDS	2.85±0.65	0.29±0.98	1.74±0.64	-0.56±1.05	<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	50.3±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

*One-way analysis of variance (ANOVA) was applied. *Mean ± SD, Those marked with the †symbol were analyzed with the Kruskal Wallis test.* ***PCOS:*** *polycystic ovary syndrome,* ***SD:*** *standard deviation,* ***BMI:*** *body mass index,* ***SDS:*** *standard deviation score,* ***SHBG:*** *Sex hormone binding globulin,* ***FAI:*** *free androgen index,* ***AMH:*** *Anti-Mullerian Hormone,* ***LH:*** *luteinizing hormone,* ***FSH:*** *follicle-stimulating hormone.*

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).

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 analyzed several biochemical variables that showed different results in PCOS diagnoses. Compared to controls, we report a higher FAI, LH/FSH ratio, AMH, and lower SHBG levels in adolescents with PCOS. The AMH has detected the best marker for PCOS diagnosis. We found higher FAI and lower SHBG levels in overweight/obese adolescents with PCOS than in the other three groups. Regardless of BMI, AMH levels were detected significantly higher in adolescents with PCOS than in healthy controls. Also, we found that the normal-weight group with PCOS had higher AMH levels than those who were obese/overweight group with PCOS. Interestingly, we demonstrated a higher LH/FSH ratio in the normal-weight group with PCOS than in the obese/overweight group with PCOS

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. AMH has been assessed for its possible use as a diagnostic criterion or auxiliary criterion for PCOS. Sahmay et al. showed that serum AMH measurement is precious for the diagnosis of women with PCOS. They detected higher serum AMH levels in women with PCOS compared to healthy controls (19). Another study from China suggested similar results. It has been reported that markers such as serum testosterone, serum AMH, LH/FSH ratio, and fasting insulin can be used in combination for accurate diagnosis of PCOS and to increase the specificity and sensitivity in the PCOS detection (20). 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 (21,22). 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 (23-25).

Until now, different AMH cutoff values with various sensitivities and specificities have been proposed, but the optimal threshold is not known. In a meta-analysis, it was reported that the AMH threshold value of 4.7 ng/mL showed specificity and sensitivity of 79.4% and 82.8%, respectively, in women with PCOS (26). Another adolescent PCOS study found that AMH level > 7.20 ng/mL showed the highest sensitivity (76.0%) and specificity (89.0%) for PCOS diagnostics in adolescence (27). Since AMH values >9 could not be measured in our study, the cut-off value for AMH could not be calculated. This is one of the limitations of this current study. Although AMH values >9 were taken as the lowest value, such as 9, we detected significantly higher serum AMH levels in the PCOS patients compared to the healthy controls which is consistent with the literature. In our study, we also demonstrated that regardless of BMI, serum AMH levels were detected significantly higher in adolescents with PCOS.

Our study found AMH levels to be higher in normal-weight patients with PCOS than in obese/overweight patients with PCOS. However, no difference was found between the AMH levels of obese and normal-weight adolescents in the healthy control group.

Despite detecting high serum AMH levels in PCOS, using different AMH test techniques and different PCOS criteria in studies cause heterogeneity between studies. As well as heterogeneity between studies, it was found significant overlap in AMH levels (28). Evidence-based recommendations from a systematic review suggested that AMH value should not be used alone as an alternative for the diagnosis of PCOS and the detection of polycystic ovary morphology (26). AMH and LH/FSH ratio combination could be useful and practical as a criterion for the diagnosis of PCOS in the presence of the PCOS criteria mentioned above. Khashchenko et al. (27) reported that LH/FSH ratio >1.23 had high sensitivity and specificity in PCOS diagnosis. In another study, the LH/FSH ratio cut-off was found to be 1.33, with lower sensitivity but higher specificity. (65.76% and 95.24%, respectively). Also serum AMH level and LH/FSH ratio were found to be similarly effective in differentiating PCOS patients from controls in this study (12). In an another study evaluating the LH/FSH ratio according to BMI (normal-high) values in patients with PCOS, no difference was found between the two groups (29). In a study comparing obese/overweight and normal weight women with different PCOS phenotypes and obese and normal weight healthy control subjects, it was shown that LH, SHBG and AMH levels were significantly lower in obese and overweight women compared to normal weight women in all groups (23). 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.

The association between SHBG levels and PCOS is limited. Obesity, an increasing problem in adolescents, increases the risk of PCOS. SHBG synthesis and secretion decrease with obesity, and this is thought to trigger PCOS by increasing the bioavailability of androgens (30). Meta-analysis of SHBG and PCOS demonstrated that SHBG levels in controls were significantly higher than those in PCOS patients, with significant heterogeneity across studies. These meta-analyses reported a significant association between low SHBG and obesity, glucose intolerance, insulin resistance, hyperandrogenism, and type 2 diabetes in women with PCOS (31). 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 (32). Moreover, another study suggested that the combination of SHBG and AMH had higher sensitivity to diagnose PCOS when compared with AMH levels alone (33). Therefore, SHBG may be a beneficial biomarker to be used in the diagnosis and post-treatment follow-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.

Most of the data in the literature revealed that compared to controls, levels of testosterone, LH, LH/FSH, and FAI were detected higher in adolescents with PCOS (12,34). The important question is which one is superior to other endocrine variables in the diagnosis of PCOS. Khashchenko et al. (27) evaluated the AMH, testosterone, FAI, androstenedione, LH/FSH ratio, ovarian volume, and ovarian-to-uterine index for PCOS prediction. In this study, it was demonstrated that using four or more of the specified criteria to diagnose PCOS, had the highest accuracy of over 90%. Moreover, they showed that the rate of correct diagnosis decreased as the number of parameters used decreased, and diagnostic precision was 85% with the use of 3 parameters (27). Another study demonstrated that AMH, LH, total testosterone, hirsutism, antral follicle count, and acanthosis nigricans are important and helpful in the diagnosis of PCOS (35). In the logistic regression model, it was shown that with four parameters (LH/FSH ratio, FAI, AMH and SHBG), 95% of PCOS cases could be detected with 87% sensitivity. The parameters that were significant in the model were the LH/FSH ratio and AMH. These parameters differed significantly in subgroup analyzes, especially in the differential diagnosis of obesity and non-obese patients.

The homogeneity of our study population and the good and clear definition of the patient and control groups constitute the strength of this study. All participants were evaluated by the same physician. As for the limitations of the study, we could not measure the AMH value greater than 9. So the AMH cut-off value could not be calculated. Also, the control and PCOS groups included a relatively low number of participants.

In conclusion, this study's results suggest that serum increased AMH, FAI, and LH/FSH ratio could be helpful and handy tests for screening adolescents with PCOS. Among them, the best marker for PCOS diagnosis was found as AMH. Moreover, AMH levels and LH/FSH ratio are negatively affected by increased adiposity in adolescents with PCOS but not healthy group. Larger studies help us to reach more precise conclusions and increase our knowledge.

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