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Anti-Müllerian Hormone and Insulin-Like Peptide 3 Levels in Adolescent Polycystic Ovary Syndrome: Correlation with Androgen Levels and Ultrasonographic Features

Polikistik Over Sendromlu Adolesanlarda Anti-Müllerian Hormon ve İnsülin Like Peptit 3 Düzeyleri: Androjen Düzeyleri ve Ultrasonografik Bulgularla İlişkisi

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ABSTRACT

Objective: Polycystic ovary syndrome (PCOS) is a common endocrine disorder characterized by chronic anovulation and hyperandrogenism. In adolescents, the diagnosis is challenging because of overlap with physiological pubertal changes. Anti-Müllerian hormone (AMH) and insulin-like peptide 3 (INSL3) are proposed biomarkers in adults, but their utility in adolescence is unclear. This study investigated serum AMH and INSL3 levels in adolescents with PCOS and healthy controls and assessed associations with ultrasonographic and biochemical features.

Methods: This cross-sectional study included 50 adolescents with PCOS (diagnosed according to the Rotterdam criteria) and 25 healthy controls. Anthropometric measurements, hormonal/biochemical assays, and transabdominal ultrasonography were performed. Correlations between biomarkers, biochemical hyperandrogenism, and ovarian morphology were evaluated. Receiver operating characteristic (ROC) analysis was performed to evaluate the diagnostic performance of AMH.

Results: Adolescents with PCOS had significantly higher serum AMH levels than controls (11.1±5.4 vs. 3.8±1.8 ng/mL; p<0.001), whereas INSL3 levels were similar (p=0.806). AMH correlated positively with total testosterone, androstenedione, free androgen index, Ferriman-Gallwey score, luteinizing hormone/follicle-stimulating hormone ratio, ovarian volume, and antral follicle count, and negatively with sex hormone-binding globulin. In the PCOS group, AMH also correlated with INSL3 (r=0.35, p=0.012), particularly in the overweight/obese subgroups. ROC analysis identified an AMH cut-off value of 5.05 ng/mL, with 94% sensitivity and 80% specificity (area under the curve: 0.938, 95% confidence interval: 0.88-0.99).

Conclusion: Serum AMH is significantly elevated in adolescents with PCOS and strongly correlates with clinical and biochemical hyperandrogenism and with ovarian morphology, suggesting its role as a supportive but not a standalone diagnostic biomarker. Unlike in adult PCOS, INSL3 showed no diagnostic value during adolescence. Incorporating AMH into clinical assessment may help identify adolescents at risk for persistent PCOS features and future metabolic complications.

Keywords: Adolescent, PCOS, AMH, INSL3, polycyctic morphology

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ÖZ

Amaç: Polikistik over sendromu (PKOS), kronik anovülasyon ve hiperandrojenizmle karakterize yaygın bir endokrin bozukluktur. Adolesanlarda, fizyolojik pubertal değişikliklerle örtüşmesi nedeniyle tanısı güçtür. Anti-Müllerian hormon (AMH) ve insülin like peptit-3 (INSL3), erişkin PKOS'ta potansiyel biyobelirteçler olarak önerilmiştir, ancak adolesan dönemdeki tanısal değerleri belirsizdir. Bu çalışmada, PKOS tanılı adolesanlarda ve sağlıklı kontrol grubunda serum AMH ve INSL3 düzeyleri araştırılmış, ultrasonografik ve biyokimyasal bulgularla ilişkileri değerlendirilmiştir.

Yöntem: Bu kesitsel çalışmaya, Rotterdam kriterlerine göre PKOS tanısı almış 50 adolesan ve 25 sağlıklı kontrol dahil edildi. Tüm katılımcılarda antropometrik ölçümler, hormonal/biyokimyasal analizler ve transabdominal ultrasonografi gerçekleştirildi. Biyobelirteçler, biyokimyasal hiperandrojenizm ve over morfolojisi arasındaki korelasyonlar değerlendirildi. AMH'nin tanısal performansı için alıcı işletim karakteristiği eğrisi (ROC) analizi yapıldı.

Bulgular: PKOS'lu adolesanlarda serum AMH düzeyleri kontrol grubuna göre anlamlı derecede yüksekti (11,1±5,4 vs. 3,8±1,8 ng/mL, p<0,001), INSL3 seviyeleri ise benzerdi (p=0,806). AMH, total testosteron, androstenedion, serbest androjen indeksi, Ferriman-Gallwey skoru, lüteinizan hormon/folikül uyarıcı hormon oranı, over hacmi ve antral folikül sayısı ile pozitif, seks hormonu bağlayıcı globulin ile negatif korelasyon gösterdi. PKOS grubunda AMH, INSL3 ile de anlamlı bir korelasyon gösterdi (r=0,35, p=0,012); özellikle fazla kilolu/obez alt gruplarında bu ilişki daha belirgindi. ROC analizi, %94 duyarlılık ve %80 özgüllük (eğri altındaki alan: 0,938, %95 güven aralığı: 0,88-0,99) ile 5,05 ng/mL'lik bir AMH kesme değeri belirledi.

Sonuç: Serum AMH, PKOS grubundaki adolesanlarda anlamlı düzeyde yüksektir ve hem klinik/biyokimyasal hiperandrojenizm hem de over morfolojisi ile güçlü bir korelasyon göstermektedir. Bu bulgu, AMH'nın tek başına tanısal bir biyobelirteç olmadığını, ancak PKOS tanısında destekleyici bir rol oynayabileceğini düşündürmektedir. Erişkin PKOS çalışmalarının aksine, adolesan dönemde INSL3 tanısal bir değer göstermemiştir. AMH'nın klinik değerlendirmeye katılması, farklı PKOS fenotiplerinin ve gelecekteki metabolik komplikasyonlar açısından risk altındaki adolesanların belirlenmesine katkı sağlayabilir.

Anahtar Kelimeler: Adolesan, PKOS, AMH, INSL3, polikistik morfoloji

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a widely recognized endocrine disorder resulting from genetic and environmental factors and characterized by diagnostic features, including chronic anovulation and hyperandrogenism confirmed clinically or biochemically. The estimated prevalence among adolescent girls worldwide is approximately 8-10%.[1,2] PCOS is increasingly recognized as a condition associated with long-term metabolic complications, including insulin resistance, type 2 diabetes mellitus, abnormal lipid profiles, and an increased risk of cardiovascular disease. Diagnosing PCOS during adolescence can be difficult because many symptoms and signs of the syndrome may resemble normal pubertal findings.[3]

There are various diagnostic guidelines and criteria. According to the Rotterdam criteria, established in 2003, PCOS is diagnosed if two or more of the following are present: ovulatory dysfunction, clinical and/or biochemical hyperandrogenism, and polycystic ovarian morphology (PCOM).[4] Diagnosis in adolescents is more challenging than in adults because androgens have different reference ranges and there are no universal thresholds; therefore, it should be evaluated according to age and pubertal stage. Physiological multicystic ovarian appearance in adolescence can be confused with PCOM; excessive reliance on pelvic ultrasound (USG) can lead to overdiagnosis. Furthermore, anovulatory cycles in the first years after menarche are physiological, and most resolve with pubertal maturation.[3,5] In 2015, the Pediatric Endocrine Society convened to establish clear criteria for diagnosing adolescent PCOS and highlighted the drawbacks of directly applying adult criteria to adolescents.[6] Diagnostic criteria may lead to unnecessary overdiagnosis, and conversely, ignoring diagnostic features may lead to delayed or missed diagnoses with unfavorable long-term consequences. The 2018 and 2023 international evidence-based PCOS guidelines emphasize the necessity of using a combination of persistent menstrual irregularity and clinical or biochemical hyperandrogenism, while discouraging the use of USG morphology as a diagnostic criterion within eight years of menarche.[7,8] Hyperandrogenism is a required criterion for the diagnosis of PCOS in adolescents. According to current guidelines, biochemical hyperandrogenism should be assessed by measuring total and free testosterone, with the free androgen index (FAI) employed to estimate free testosterone levels.[8]

Anti-Müllerian hormone (AMH) is a glycoprotein of the transforming growth factor-β family, secreted predominantly by granulosa cells in preantral and small antral follicles of the ovaries in women. Because it is stable across the menstrual cycle and directly indicates ovarian reserve, AMH is commonly used in reproductive medicine to evaluate ovarian reserve, predict menopause, guide infertility treatment, and support the diagnosis of PCOS.[9-11] In individuals with PCOS, AMH concentrations are generally elevated, reflecting the increased caunt of preantral and small antral follicles. However, in adolescents, the abundance of physiological follicles and pubertal variability limit its diagnostic utility.

Insulin-like peptide 3 (INSL3), a new member of the insulin/relaxin family, is secreted by theca interna cells within antral follicles and by the corpus luteum and ovarian stromal tissue. It is thought to be associated with follicle number and development.[12] Several studies have demonstrated that INSL3 levels are higher in adults with PCOS and that

they are associated with antral follicle number.[13] However, studies of adolescents are limited, and available evidence suggest that INSL3 does not provide significant diagnostic value for PCOS in this age group.[14]

While current guidelines support the use of PCOM and AMH for diagnosing adult PCOS, using PCOM and AMH is not recommended in the diagnosis of adolescent PCOS.[5,8] However, it is important to identify adolescents who do not fully meet the diagnostic criteria but are predisposed to PCOS and its chronic complications. Our study aimed to evaluate AMH and INSL3 concentrations in adolescents with PCOS and healthy controls, and to compare these concentrations with ultrasonographic and biochemical findings. It was hypothesized that serum AMH and INSL3 would be elevated in adolescents diagnosed with PCOS and would show positive correlations with ultrasonographic findings and androgen levels.

METHODS

Study Population

Fifty adolescent girls aged 12-18 years who were admitted to the Hacettepe University, Division of Pediatric Endocrinology, between July 2014 and March 2015, participated in the study. PCOS was diagnosed in accordance with the Rotterdam consensus, which requires at least two of the three diagnostic criteria: oligo/anovulation, clinical or biochemical hyperandrogenism, and PCOM.[4] Participants with comorbidities, such as chronic systemic disease, thyroid abnormalities, hyperprolactinemia, congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, or other endocrine disorders, or with prior treatment for PCOS, were excluded.

Twenty-five healthy adolescent girls with regular menstrual cycles for at least two years were selected as controls. Menstrual cycles were considered eumenorrheic when the interval ranged between 21 and 45 days, oligomenorrheaic when the interval exceeded 45 days or there were fewer than eight menstrual episodes within a year, and amenorrheaic when the absence of menses persisted more than three months.[5] Only participants who were at least two years post-menarche were enrolled in the study. Age at menarche and family history of PCOS were recorded. Written informed consent was obtained from both the adolescents and their parents.

Clinical Evaluation

Anthropometric Evaluation

Weight and height assessments were carried out by a single physician (A.K.T) using a calibrated wall-mounted Harpenden stadiometer, and an electronic scale (sensitivity 0.1 kg) after 12 hours of fasting, barefoot, and wearing normal clothing. The body mass index (BMI) was calculated using the formula: weight (kg)/height^2 (m^2). Participants were grouped by BMI percentile: 5th-85th percentile as normal weight, 85th-95th percentile as overweight, and ≥95th percentile as obese. Standard deviation scores (SDS) for anthropometric parameters were computed.[15] Patients were only eligible for enrollment if at least two years had elapsed since menarche. A detailed medical history and systemic physical examination were obtained from all participants by the same physician (A.K.T).

Assessment of Clinical Hyperandrogenism

Clinical hyperandrogenism was assessed according to the Ferriman-Gallwey scoring system. A score of ≥8 was accepted as clinical hyperandrogenism.[16]

Laboratory Evaluation

Blood Sampling

Blood samples were collected from all participants on days 2-3 of the menstrual cycle, corresponding to the early follicular phase. In patients with amenorrhea, samples were collected after a 12-hour overnight fast on the day of the clinical evaluation at 08:00 AM. Serum was promptly separated, frozen, and preserved at -80 °C until analysis.

Biochemical Analyses

Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), total testosterone, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), androstenedione, 17-hydroxyprogesterone (17OHP), AMH, and INSL3 were measured in all patients with PCOS and in controls. To exclude differential diagnoses, basal 17OHP levels were assessed for adrenal enzymatic abnormalities, free thyroxine (T4) and thyroid-stimulating hormone (TSH) for thyroid dysfunction, and prolactin for hyperprolactinemia. Serum FSH, LH, E2, prolactin, TSH, and free T4 were measured using a two-step chemiluminescence microparticle immunoassay; SHBG and 17OHP by immunoradiometric assay; DHEAS and total testosterone by solid-phase chemiluminescence immunoassay; and AMH and INSL3 by enzyme-linked immunosorbent assay (Beckman Coulter®, Webster USA; Elabscience®, USA)

FAI was calculated using the formula: [total testosterone (nmol/L) ÷ SHBG (nmol/L)] × 100. Serum testosterone levels measured in ng/dL were converted to nmol/L using the conversion factor 1 ng/dL = 0.0347 nmol/L.

Pelvic Ultrasound

On the day of hormonal and biochemical assessments, pelvic USG examinations were also conducted.

Prospective transabdominal USG scans were performed in both the PCOS and control groups by a pediatric radiologist (H.N.Ö), who was blinded to the clinical and laboratory information of the participants. Imaging was conducted using a Sonoline Elegra US scanner (Siemens, Erlangen, Germany) with a 2-5 MHz convex-array broadband transducer in the department of radiology.

For each subject, uterine dimensions, endometrial thickness, and ovarian dimensions [longitudinal, transverse, and anteroposterior (AP) diameters] were measured in the sagittal and coronal planes. Uterine and ovarian volumes were computed according to the ellipsoid formula: length × AP diameter × transverse diameter × π/6. The follicles were grouped by size into three categories: <5 mm, 6-10 mm, and >10 mm. Follicle counts were documented for each group. Antral follicles (<10 mm) were counted, and cysts (>10 mm) were noted. PCOM was described by the presence of ≥12 follicles measured <10 mm, and/or ovarian volume >10 mL.[4,17]

Statistical Analysis

Statistical analyses were performed using SPSS version 21 (Chicago, IL, USA). Data are given as mean ± standard deviation (SD) or as median (minimum-maximum). Normality was examined with the Kolmogorov-Smirnov test. Categorical data are expressed as percentages (%). Group comparisons were made using Student's t-test or Mann-Whitney U test. Categorical data were analyzed with the chi-square test. Correlations were assessed using Pearson's correlation for normally distributed variables and Spearman's rank correlation when at least one variable was not normally distributed. A p-value <0.05 was accepted as statistically significant.

Biomarkers demonstrating significant differences between groups were further evaluated using receiver operating characteristic (ROC) curve analysis to determine their diagnostic values (sensitivity and specificity). An area under the curve (AUC) was considered statistically significant at a type 1 error level of <5%.

Ethical Approval

The study was approved by the Ethics Committee of the Faculty of Medicine at Hacettepe University (approval no: GO 14/256-12, date: 04.06.2014) Written informed consent was obtained from all participants and their parents.

RESULTS

50 adolescents with PCOS and 25 healthy controls were included in the analysis. The mean age and age at menarche were similar among the groups (p>0.05). Compared to controls, adolescents with PCOS had significantly higher weight SDS (p=0.003) and BMI SDS (p<0.001), whereas height SDS were similar between groups (Table 1). All participants in the control group had normal BMI values, whereas approximately half of the adolescents in PCOS group were overweight or obese.

Serum LH concentrations and the LH/FSH ratio were significantly elevated in adolescents with PCOS (LH: 10.47±6.29 vs. 6.53±4.98 mIU/mL, p=0.008; LH/FSH ratio: 1.83±1.07 vs. 1.22±1.11, p=0.025), whereas FSH levels were similar (p=0.454). The PCOS group had significantly elevated levels of serum total testosterone (64.04±21.43 vs. 27.91±9.64 ng/mL; p<0.001), DHEAS (275.66±114.35 vs. 170.09±89.98 µg/dL; p<0.001), and androstenedione (4.65±2.36 vs. 1.93±0.79 ng/mL; p<0.001) compared with controls. Additionally, the FAI was significantly elevated in PCOS cases (p<0.001). Serum SHBG and E2 concentrations were similar between groups. 17OHP levels were also significantly elevated in adolescents with PCOS (p<0.001) (Table 1).

Serum AMH levels were significantly higher in the PCOS group (11.1±5.42 ng/mL) than in the controls (3.76±1.75 ng/mL) (p<0.001). INSL3 levels were similar across groups (p=0.806). Anthropometric, clinical, and laboratory data of the PCOS and control groups are presented in Table 1. The mean ovarian volume was significantly greater in the PCOS group compared with the control group (13.99±5.22 vs. 9.70±3.64 mL, p<0.001). Both the right and left ovarian volumes were significantly higher in adolescents with PCOS (right: 14.76±6.69 vs. 10.60±4.78 mL, p=0.003; left: 13.22±5.29 vs. 8.75±3.80 mL, p<0.001). The number of follicles <5 mm and the overall antral follicle number were significantly higher in PCOS cases than in controls (both p<0.001) (Table 2). Adolescents with amenorrhea in the PCOS group had significantly higher antral follicle counts (AFCs) than those without amenorrhea (p<0.001).

Normal ovarian morphology was observed in 5 (10%) of the PCOS group, whereas PCOM was observed in 3 (12%) of the control group. Ultrasonographic features of patients with PCOS and controls are summarized in Table 2.

Correlation Analysis

Correlation analysis showed significant positive correlations between serum AMH concentrations and LH (r=0.462, p<0.001), LH/FSH ratio (r=0.448, p<0.001), total testosterone (r=0.590, p<0.001), androstenedione (r=0.524, p<0.001), FAI (r=0.315, p=0.006), Ferriman-Gallwey score (r=0.490, p<0.001), total ovarian volume (r=0.496, p<0.001), and AFC (r=0.620, p<0.01). In contrast, AMH was negatively correlated with SHBG (r=-0.241, p=0.037).

No significant correlations were detected between INSL3 and other hormonal and ultrasonographic parameters. However, in the PCOS group, AMH showed a moderate

positive correlation with INSL3 (r=0.35, p=0.012). Subgroup analysis according to BMI demonstrated that this correlation was stronger in overweight patients (r=0.532, p=0.050) and obese patients (r=0.595, p=0.032).

Diagnostic Value of Serum Anti-Müllerian Hormone Level

ROC curve analysis was used to assess the diagnostic ability of AMH in adolescents with PCOS (Figure 1). At a cut-off level of 5.05 ng/mL, AMH demonstrated a sensitivity

Table 1. Anthropometric, clinical, and laboratory characteristics of adolescents with PCOS and control group

Parameter	PCOS (n=50)	Controls (n=25)	p
Age (years)	15.9±1.13	15.7±1.21	0.362
Age at menarche (years)	12.2±0.11	12.4±1.25	0.546
Weight (kg)	66.76±17.02	54.44±7.6	<0.001
Weight SDS	0.77±1.18	-0.02±0.79	0.003
Height (cm)	160.1±6.3	161.7±7.16	0.342
Height SDS	-0.32±0.93	-0.74±1.07	0.251
BMI (kg/m^2)	25.9±6.08	20.7±2.23	<0.001
BMI SDS	1.16±1.74	-0.31±1.12	<0.001
Ferriman-Gallwey score	16.14±6.7	4.92±2.06	<0.001
FSH (mIU/mL)	6.11±2.39	5.72±1.99	0.454
LH (mIU/mL)	10.47±6.29	6.53±4.98	0.008
LH/FSH ratio	1.83±1.07	1.22±1.11	0.025
Total testosterone (ng/dL)	64.04±21.43	27.91±9.64	<0.001
DHEAS (µg/dL)	275.66±114.35	170.09±89.98	<0.001
Androstenedione (ng/mL)	4.65±2.36	1.93±0.79	<0.001
SHBG (nmol/L)	37.98±36.41	45.30±18.57	0.253
Estradiol (pg/mL)	50±30	48±30	0.798
17OHP (ng/mL)	1.47±0.73	0.89±0.44	<0.001
FAI	11.20±10.51	2.98±2.96	<0.001
AMH (ng/mL)	11.1±5.42	3.76±1.75	<0.001
INSL3 (ng/mL)	0.23±0.18	0.22±0.15	0.806

Values are presented as mean ± standard deviation. DHEAS: Dehydroepiandrosterone sulfate, SHBG: Sex hormone-binding globulin, 17OHP: 17-hydroxyprogesterone, AMH: Anti-Müllerian hormone, INSL3: Insulin-like peptide-3, PCOS: Polycystic ovary syndrome, BMI: Body mass index, SDS: Standard deviation scores, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone. FAI: Free androgen index, calculated as [total testosterone (nmol/L) ÷ SHBG (nmol/L)] × 100

Table 2. USG features of PCOS and control group

Parameter	PCOS (n=50)	Controls (n=25)	p
Mean ovarian volume (mL)	13.99±5.22	9.70±3.64	<0.001
Left ovarian volume (mL)	13.22±5.29	8.75±3.80	<0.001
Right ovarian volume (mL)	14.76±6.69	10.60±4.78	0.003
Endometrial thickness (mm)	6.63±2.39	5.78±3.04	0.226
Number of follicles <5 mm (count, per ovary)	10.00±4.42	3.14±2.76	<0.001
Number of follicles 6-10 mm (count, per ovary)	3.42±4.66	4.16±2.22	0.450
Antral follicles <10 mm (count, per ovary)	13.42±4.6	7.30±2.28	<0.001

Data are presented as mean ± standard deviation. "Per-ovary" mean = (right + left)/2
PCOS: Polycystic ovary syndrome, USG: Ultrasound

of 94% and a specificity of 80%. The AUC was 0.938 [95% confidence interval (CI): 0.88-0.99, p<0.001].

DISCUSSION

PCOS is one of the most frequently diagnosed endocrine disorders in adolescent females and continues to pose a diagnostic challenge owing to the overlap between physiological pubertal changes and the early manifestations of the syndrome.[1] In our adolescent cohort diagnosed with PCOS according to the Rotterdam criteria, serum AMH levels were significantly elevated compared with those of their healthy peers. This finding, although not included in current diagnostic criteria, highlights the potential role of AMH as a supportive marker of PCOS in adolescents.

INSL3 and AMH are produced by granulosa and theca cells in mammalian gonads.[10,12] In relation with the elevated preantral and antral follicle caunts in PCOS, studies showed that AMH is widely reported in individuals with PCOS, especially in adults.[14,18,19] In line with our results, previous studies have consistently reported elevated AMH concentrations in adolescents with PCOS. In a recent meta-analysis including 15 studies, Tsukui et al.[20] showed that serum AMH concentrations were significantly higher in adolescents with PCOS than in controls, with an average

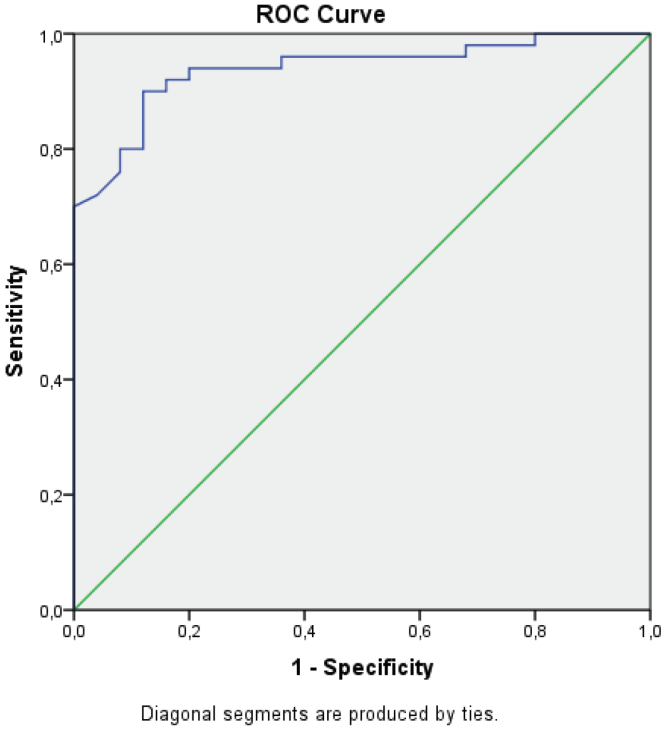

Figure 1. ROC curve of serum anti-Müllerian hormone levels in adolescents with polycystic ovary syndrome. The curve plots sensitivity (y-axis) against 1-specificity (x-axis)

ROC: Receiver operating characteristic

weighted difference of 2.91 ng/mL (95% CI: 0.74-5.09). In the same study, AFC was also significantly higher in the PCOS group, with a mean difference of 7.14 follicles (95% CI: 2.70-11.59). In our study, a cut-off level of 5.05 ng/mL was determined for serum AMH in adolescents with PCOS, demonstrating 94% sensitivity and 80% specificity. An examination of cut-off values reported in adolescent PCOS studies in the literature reveals that they are generally within the range of 5.8-7.25 ng/mL, although some studies have reported higher (≥8-10 ng/mL) or lower values (Table 3).[14,21-29] Although the cut-off value obtained in this study is at the lower end of this range, it is particularly notable for its high sensitivity. Differences between studies are thought to result from measurement kits, variations in diagnostic criteria, sample characteristics (obesity, time since menarche, ethnic distribution), and study designs. In contrast to our findings, Kocaay et al.[30] found no difference in AMH levels among adolescent PCOS cases and the control group. Despite these differences, the findings suggest that AMH is a valuable complementary marker for diagnosing PCOS in adolescents and may increase its diagnostic power, especially when evaluated together with androgen levels and ultrasonographic findings.

Data in the literature on the relationship between serum AMH and androgen levels are contradictory. Although the majority of studies exhibited a positive correlation among AMH and androgen concentrations, some other studies had shown no correlation.[31-34] In this study, AMH was significantly correlated with androgens (including total testosterone and androstenedione), as well as with FAI and the Ferriman-Gallwey score, suggesting that AMH may also reflect the degree of clinical and biochemical hyperandrogenism in adolescents with PCOS.

The FAI, defined as total testosterone divided by SHBG, provides an estimate of free testosterone. It is recommended by current guidelines as a diagnostic marker for biochemical hyperandrogenism.[7] In their study on adolescents, Sağsak et al.[35] showed that individuals with an FAI above 6.15 warrant evaluation for PCOS. Villarroel et al.[36] study on adolescent PCOS also found that an FAI ≥6.1% is valuable for diagnosing PCOS. On the other hand Özer et al.[37] found that FAI measurements were found similar among adolescents with PCOS and hyperinsulinemia/obesity, while SHBG levels were lower in hyperinsulinemia/obesity adolescents with oligomenorrhea. These findings suggest that neither FAI nor SHBG alone is a reliable diagnostic marker for PCOS in the presence of metabolic disorders. Several studies have also demonstrated the diagnostic utility of FAI, reporting higher levels in adolescents with PCOS compared to healthy controls.[14,28] In our study, no difference in SHBG levels was identified among the PCOS group and controls,

Table 3. AMH cut-off values for adolescent PCOS determined by ROC analysis in the literature

Author-year (ref)	AMH cut-off (ng/mL)	AUC	95% CI	Sensitivity	Specificity	p value
Li et al.[21]	8 ng/mL	0.664	0.551-0.778	70%	61.7%	
Deveer et al.[22]	6.66 ng/mL	0.820		62%	76%	
Tokmak et al.[23]	7.11 ng/mL	0.763	0.607-0.920	84%	66.7%	0.004
Yetim et al.[14]	6.1 ng/mL	0.880	0.800-0.960	81.1%	92.3%	<0.001
Savas-Erdeve et al.[24]	7.25 ng/mL	0.700	0.591-0.808	58%	72.5%	0.001
Kim et al.[25]	6.26 ng/mL	0.788	0.687-0.868	67%	81%	<0.001
Merino et al.[26]	7.03 ng/mL	0.758		58.8%	82.1%	0.0001
Yetim Şahin et al.[27]	10 ng/mL	0.326	0.171-0.482	50%	69%	0.047
Khashchenko et al.[28]	7.20 ng/mL			76%	89%	
Tunç and Özkan[29]	5.8 ng/mL			86.9%	70%	
Kömürlüoğlu Tan (present study)	5.05 ng/mL	0.938	0.880-0.990	94%	80%	<0.001

AMH: Anti-Müllerian hormone, AUC: Area under the curve, CI: Confidence interval, ROC: Receiver operating characteristic, PCOS: Polycystic ovary syndrome

whereas FAI was significantly elevated in adolescents in the PCOS group compared with controls. These results are consistent with the literature and suggest that FAI can be used as a supportive marker in adolescent PCOS. However, due to differences in pubertal stage, obesity, and measurement methods, FAI alone is not diagnostic and should be interpreted in conjunction with other clinical and biochemical findings.

Özer et al.[37] found the LH/FSH ratio was also determined as a predictive marker for PCOS. Previous studies have consistently reported elevated LH/FSH ratios in adolescents diagnosed PCOS, supporting our findings of higher ratios in this population.[14,28] In our cohort, the LH/FSH ratio was significantly elevated in PCOS cases than in controls, and AMH was correlated positively with the LH/FSH ratio ($r=0.448$; $p<0.01$). Since the LH/FSH ratio is variable during adolescence, it does not provide diagnosis alone; it is best to interpret this ratio together with high FAI and ovarian USG findings or AMH.

Previous studies have reported a potential role for INSL3 in the pathogenesis of PCOS. Data on INSL3 levels in adolescent PCOS are limited compared with those from studies in adults. In adult women with PCOS, elevated INSL3 concentrations have consistently been reported, reflecting increased theca cell activity.[12] In this study, Gambineri et al.[12] also reported significantly higher INSL3 levels in adult women with PCOS, with positive correlations to androgen concentrations. Similarly, Pelusi et al.[38] found that INSL3 was associated with androgen levels in adolescents with anovulatory cycles. These findings indicate that INSL3 may reflect ovarian steroidogenic activity and hyperandrogenism at an early stage. However, in our cohort, INSL3 levels did not differ among adolescents with PCOS and controls, and were not associated with ultrasonographic features or androgen levels, suggesting that its diagnostic utility in adolescence remains limited. Similar to our results Tunç and Özkan[29] and Yetim et al.[14] found INSL3 levels to be similar to the control group in their study on adolescent PCOS patients, and did not detect any correlation with any laboratory or clinical parameters. These discrepancies between adolescent and adult populations may be due to theca cell activity and INSL3 secretion not yet being fully established during early adolescence, resulting in a weaker association with androgenic and morphological traits. Furthermore, differences in analysis methodologies and sample sizes across studies may contribute to the inconsistent findings. When considered together, these findings indicate that INSL3 may be a biomarker of hyperandrogenism in adults, but its role in adolescents appears less reliable and requires further investigation in larger longitudinal studies.

Pelusi et al.[13] demonstrated a significant association between AMH and INSL3 levels in their study of adult women with PCOS, particularly among those with amenorrhea. Similarly, we found a significant correlation between AMH and INSL3 in adolescents with PCOS; this association was particularly evident in overweight/obese subgroups. This may reflect the combined effect of metabolic and gonadal factors on ovarian function and suggest that body composition may modulate the relationship between AMH and INSL3. These results support the hypothesis that INSL3 and AMH are co-regulated and suggest that they provide a complementary perspective on the pathophysiology of PCOS.

Many studies of adult women have shown a positive association among serum AMH levels and follicle counts in PCOS patients, reflecting increased AMH production

resulting from the elevated preantral and AFCs in polycystic ovaries.[39,40] Findings in adolescents have been less consistent. Pawelczak et al.[34] demonstrated a positive association among AMH, ovarian volume and peripheral follicle distribution. Similary, Villarroel et al.[36] noted a positive link between among AMH and the caunt of 2-5 mm follicles. In contrast, Yetim et al.[14] showed no correlation among AMH and ultrasonographic features, and Savas-Erdeve et al.[24] also documented no significant association among AMH, ovarian volume, and number of follicles. In our study, serum AMH was positively correlated with both ovarian volume and antral follicle number in adolescent girls with PCOS, supporting its role as a marker reflecting ovarian morphology in this population.

Follicle counts are physiologically high and variable in adolescents, so they are unreliable in diagnosing PCOS. Current guidelines do not recommend pelvic USG for diagnostic purposes before 8 years postmenarche.[7] If USG is used during this period, ovarian volume should be the preferred morphological criterion rather than the follicle count. Previous studies have reported that mean ovarian volumes in adolescent patients with PCOS were higher than those in healthy individuals.[24,28] In our cohort, mean ovarian volumes were significantly greater in adolescents with PCOS than in controls (right ovary: 14.8 vs. 10.6 cm^3; left ovary: 13.2 vs. 8.8 cm^3), supporting ovarian volume as a more informative morphological indicator in this age group.

The relationship between obesity and serum AMH levels is controversial. Obesity may affect serum AMH concentrations through several metabolic and inflammatory mechanisms. Studies in adult women with PCOS have reported lower AMH levels due to leptin elevation, insulin resistance, and impaired follicular development secondary to chronic inflammation.[41,42] In an adolescent study conducted in our country, normal-weight adolescents with PCOS exhibited higher AMH levels than their overweight or obese peers.[43] Conversely, other studies have reported elevated AMH levels in obese adolescents with PCOS.[25,44] In our study, no significant differences in AMH levels were found among normal-weight, overweight, and obese participants. These conflicting findings suggest that the relationship among obesity and AMH is complex and can be affected by age-related, environmental, hormonal, and metabolic factors.

Although AMH is not currently recommended as a diagnostic criterion in adolescents due to test variability and the physiological changes of puberty, our findings highlight its potential clinical utility and suggest that AMH can be considered a supportive criterion for PCOS. In our cohort, AMH showed strong correlations not only with AFC and ovarian volume, but also with biochemical and clinical hyperandrogenism (testosterone, androstenedione levels, FAI, and Ferriman-Gallwey score) and with the LH/FSH ratio. These findings suggest that AMH reflects both the morphological and endocrine features of PCOS. The high sensitivity of the 5.05 ng/mL cut-off value suggests that AMH may serve as a supportive biomarker for identifying adolescents at risk of persistent PCOS features. It has also been shown in the literature that high AMH in adolescence may be associated with future development of PCOS.[31] However, due to its limited specificity and lack of test standardization, AMH should not be used alone but in conjunction with established clinical and biochemical criteria.

Study Limitations

This study is valuable because it enables objective comparison of findings between adolescents with PCOS and healthy controls using standardized pelvic USG performed by a blinded pediatric radiologist. Simultaneously measured AMH, INSL3, gonadotropins, and androgen levels were correlated with detailed ultrasonographic measurements. We demonstrated robust diagnostic performance of AMH in adolescents (cut-off point of 5.05 ng/mL; AUC of 0.938; sensitivity of 94%; specificity of 80%) and consistent positive correlations between AMH and biochemical and clinical hyperandrogenism (total testosterone, androstenedione, FAI, Ferriman-Gallwey score), the LH/FSH ratio, and mean ovarian volumes. In contrast to data from adults, we found that INSL3 did not distinguish adolescents with PCOS from controls. These results indicate that biomarker levels and their interpretation, in addition to hyperandrogenism and oligo/amenorrhea criteria, can contribute significantly to the clinical decision-making process for diagnosing adolescent PCOS.

This study has some limitations. First, applying the Rotterdam criteria derived from adults to adolescents carries the risk of misclassification because anovulatory cycles are common in the early postmenarche period, and physiologically high or variable follicle counts may exaggerate polycystic morphology, potentially leading to overdiagnosis. Transabdominal USG, used because transvaginal USG, is unsuitable for virgin adolescents, may underestimate follicle count; to mitigate this limitation, we prioritized ovarian volume as a morphological descriptor. The limited sample size and the cross-sectional, single-center design restrict causal inference and the generalizability of the findings. Finally, we were unable to perform longitudinal follow-up to determine the transition of adolescent phenotypes into adulthood and did not consider all metabolic confounders (e.g., insulin resistance) in subgroup analyses.

CONCLUSION

In this adolescent cohort, serum AMH levels were significantly elevated in the PCOS group compared with controls, and correlated with ovarian morphology (ovarian volume, AFC), biochemical/clinical hyperandrogenism (total testosterone, androstenedione, FAI, Ferriman-Gallwey score), and the LH/FSH ratio. FAI was significantly elevated in PCOS, whereas INSL3 did not distinguish adolescents with PCOS from controls. These findings support the use of AMH as an adjunct test in the diagnosis of PCOS in adolescence, rather than as a standalone parameter. AMH may be particularly helpful for identifying at-risk adolescents prior to a definitive PCOS diagnosis and for the early stratification of future metabolic risk. Given test variability and limited specificity, AMH should be considered together with clinical data and other biochemical assessments. Larger, prospective multicenter studies are required to biomarkers in adolescent PCOS cases and to evaluate assess the long-term metabolic consequences of these biomarkers.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of the Faculty of Medicine at Hacettepe University (approval no: GO 14/256-12, date: 04.06.2014).

Informed Consent: Written informed consent was obtained from all participants and their parents.

Footnotes

Authorship Contributions

Concept: A.K.T., E.N.G., A.A., Design: A.K.T., Z.A.Ö., N.K., A.A., Data Collection or Processing: A.K.T., H.N.Ö., Analysis or Interpretation: A.K.T., E.N.G., Z.A.Ö., N.K., A.A., Literature Search: A.K.T., H.N.Ö., A.A., Writing: A.K.T.

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REFERENCES

1. Ibáñez L, de Zegher F. Polycystic ovary syndrome in adolescent girls. Pediatr Obes. 2020;15:e12586.
2. Teede HJ, Misso ML, Costello MF, et al; International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod. 2018;33:1602-18.
3. Ibáñez L, Oberfield SE, Witchel S, et al. An international consortium update: pathophysiology, diagnosis, and treatment of polycystic ovarian syndrome in adolescence. Horm Res Paediatr. 2017;88:371-95.
4. 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). Hum Reprod. 2004;19:41-7.
5. Peña AS, Witchel SF, Hoeger KM, et al. Adolescent polycystic ovary syndrome according to the international evidence-based guideline. BMC Med. 2020;18:72.
6. Witchel SF, Oberfield S, Rosenfield RL, et al. The Diagnosis of Polycystic Ovary Syndrome during Adolescence. Horm Res Paediatr. 2015.
7. Peña AS, Witchel SF, Boivin J, et al. International evidence-based recommendations for polycystic ovary syndrome in adolescents. BMC Med. 2025;23:151.
8. Teede HJ, Tay CT, Laven JJE, et al. Recommendations from the 2023 International Evidence-based Guideline for the Assessment and Management of Polycystic Ovary Syndrome. J Clin Endocrinol Metab. 2023;108:2447-69.
9. Iwase A, Hasegawa Y, Tsukui Y, et al. Anti-Müllerian hormone beyond an ovarian reserve marker: the relationship with the physiology and pathology in the life-long follicle development. Front Endocrinol (Lausanne). 2023;14:1273966.
10. Weenen C, Laven JS, Von Bergh AR, et al. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod. 2004;10:77-83.
11. Leader B, Baker VL. Maximizing the clinical utility of antiMüllerian hormone testing in women's health. Curr Opin Obstet Gynecol. 2014;26:226-36.
12. Gambineri A, Patton L, De lasio R, Palladoro F, Pagotto U, Pasquali R. Insulin-like factor 3: a new circulating hormone related to luteinizing hormone-dependent ovarian hyperandrogenism in the polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92:2066-73.
13. Pelusi C, Fanelli F, Pariali M, Zanotti L, Gambineri A, Pasquali R. Parallel variations of insulin-like peptide 3 (INSL3) and antimüllerian hormone (AMH) in women with the polycystic ovary syndrome according to menstrual cycle pattern. J Clin Endocrinol Metab. 2013;98:E1575-82.
14. Yetim A, Yetim Ç, Baş F, et al. Anti-Müllerian hormone and inhibin-a, but not inhibin-b or insulin-like peptide-3, may be used as surrogates in the diagnosis of polycystic ovary syndrome in adolescents: preliminary results. J Clin Res Pediatr Endocrinol. 2016;8:288-97.
15. Neyzi O, Bundak R, Gökçay G, et al. Reference values for weight, height, head circumference, and body mass index in Turkish children. J Clin Res Pediatr Endocrinol. 2015;7:280-93.
16. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab. 1961;21:1440-7.
17. Fulghesu AM, Canu E, Casula L, Melis F, Gambineri A. Polycystic ovarian morphology in normocyclic non-hyperandrogenic adolescents. J Pediatr Adolesc Gynecol. 2021;34:610-6.
18. Dewailly D, Gronier H, Poncelet E, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod. 2011;26:3123-9.
19. Woo HY, Kim KH, Rhee EJ, Park H, Lee MK. Differences of the association of anti-Müllerian hormone with clinical or biochemical characteristics between women with and without polycystic ovary syndrome. Endocr J. 2012;59:781-90.
20. Tsukui Y, Kitahara Y, Hasegawa Y, Kobayashi M, Osuka S, Iwase A. Anti-Müllerian hormone levels in the diagnosis of adolescent

polycystic ovarian syndrome: a systematic review and meta-analysis. Endocr J. 2022;69:897-906.

21. Li L, Chen X, Mo Y, Chen Y, Wenig M, Yang D. Elevated serum antiMullerian hormone in adolescent and young adult Chinese patients with polycystic ovary syndrome. Wien Klin Wochenschr. 2010;122:519-24.
22. Deveer M, Deveer R, Basaran O, et al. Serum copeptin, pentraxin 3, antiMüllerian hormone levels with echocardiography and carotid artery intima-media thickness in adolescents with polycystic ovary syndrome. J Clin Med Res. 2015;7:989-94.
23. Tokmak A, Kokanali D, Timur H, Kuntay Kokanali M, Yilmaz N. Association between antiMüllerian hormone and insulin resistance in non-obese adolescent females with polycystic ovary syndrome. Gynecol Endocrinol. 2016;32:926-30.
24. Savas-Erdeve S, Keskin M, Sagsak E, Cenesiz F, Cetinkaya S, Aycan Z. Do the Anti-Müllerian hormone levels of adolescents with polycystic ovary syndrome, those who are at risk for developing polycystic ovary syndrome, and those who exhibit isolated oligomenorrhea differ from those of adolescents with normal menstrual cycles? Horm Res Paediatr. 2016;85:406-11.
25. Kim JY, Tfayli H, Michaliszyn SF, Lee S, Nasr A, Arslanian S. Anti-Müllerian hormone in obese adolescent girls with polycystic ovary syndrome. J Adolesc Health. 2017;60:333-9.
26. Merino PM, Villarroel C, Jesam C, López P, Codner E. New diagnostic criteria of polycystic ovarian morphology for adolescents: impact on prevalence and hormonal profile. Horm Res Paediatr. 2017;88:401-7.
27. Yetim Şahin A, Baş F, Yetim Ç, et al. Determination of insulin resistance and its relationship with hyperandrogenemia,anti-Müllerian hormone, inhibin A, inhibin B, and insulin-like peptide-3 levels in adolescent girls with polycystic ovary syndrome. Turk J Med Sci. 2019;49:1117-25.
28. Khashchenko E, Uvarova E, Vysokikh M, et al. The relevant hormonal levels and diagnostic features of polycystic ovary syndrome in adolescents. J Clin Med. 2020;9:1831.
29. Tunç S, Özkan B. Analysis of new biomarkers for the diagnosis of polycystic ovary syndrome in adolescents. J Curr Pediatr. 2021;19:311-8.
30. Kocaay P, Siklar Z, Buyukfirat S, Berberoglu M. The diagnostic value of anti-Müllerian hormone in early post menarche adolescent girls with polycystic ovarian syndrome. J Pediatr Adolesc Gynecol. 2018;31:362-6.
31. Pinola P, Morin-Papunen LC, Bloigu A, et al. Anti-Müllerian hormone: correlation with testosterone and oligo- or amenorrhoea in female adolescence in a population-based cohort study. Hum Reprod. 2014;29:2317-25.
32. Piouka A, Farmakiotis D, Katsikis I, Macut D, Gerou S, Panidis D. AntiMüllerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: relationship with increased luteinizing hormone levels. Am J Physiol Endocrinol Metab. 2009;296:E238-43.
33. Siow Y, Kives S, Hertweck P, Perlman S, Fallat ME. Serum Müllerian-inhibiting substance levels in adolescent girls with normal menstrual cycles or with polycystic ovary syndrome. Fertil Steril. 2005;84:938-44.
34. Pawelczak M, Kenigsberg L, Milla S, Liu YH, Shah B. Elevated serum anti-Müllerian hormone in adolescents with polycystic ovary syndrome: relationship to ultrasound features. J Pediatr Endocrinol Metab. 2012;25:983-9.
35. Sağsak E, Keskin M, Çetinkaya S, Erdeve ŞS, Aycan Z. The diagnostic value of free androgen index in obese adolescent females with idiopathic hirsutism and polycystic ovary syndrome. J Acad Res Med. 2021;11:81-5.
36. Villarroel C, Merino PM, López P, et al. Polycystic ovarian morphology in adolescents with regular menstrual cycles is associated with elevated antiMüllerian hormone. Hum Reprod. 2011;26:2861-8.
37. Özer E, Taş D, Çakır Gündoğan S, Boyraz M, Gürbüz F. Clinical utility of FAI and SHBG in differentiating PCOS from anovulatory cycles in adolescent girls. Front Pediatr. 2025;13:1581060.
38. Pelusi C, Stancampiano M, Fanelli F, Pariali M, Gambineri A, Pasquali R. Anti-müllerian hormone and insulin-like 3 levels in healthy normal-weight ovulatory and anovulatory eumenorrheic late adolescent females: potential early biomarkers of ovarian dysfunction? Eur J Obstet Gynecol Reprod Biol. 2015;195:188-92.
39. Pigny P, Merlen E, Robert Y, et al. Elevated serum level of antiMüllerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. J Clin Endocrinol Metab. 2003;88:5957-62.
40. Fanchin R, Schonäuer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Müllerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprod. 2003;18:323-7.
41. Zhao H, Zhou D, Liu C, Zhang L. The relationship between insulin resistance and obesity and serum antiMüllerian hormone level in Chinese women with polycystic ovary syndrome: a retrospective, single-center cohort study. Int J Womens Health. 2023;15:151-66.
42. Bernardi LA, Carnethon MR, de Chavez PJ, et al. Relationship between obesity and anti-Müllerian hormone in reproductive-aged African American women. Obesity (Silver Spring). 2017;25:229-35.
43. Büyükyılmaz G, Koca SB, Toksoy Adıgüzel K, Boyraz M, Gurbuz F. The role of the AMH, SHBG, free androgen index and LH/FSH ratio in the diagnosis of polycystic ovary syndrome in adolescent. Turkish J Pediatr Dis. 2024;18:34-40.
44. Shrikhande BA, Shrikhand LA. Association between weight loss in obese Indian adolescent girls with polycystic ovarian syndrome and decline in anti-Müllerian hormone concentrations. J South Asian Feder Obst Gynaecol. 2019;11:110-2.