ORIGINAL ARTICLE ÖZGÜN ARAŞTIRMA

Analysis of New Biomarkers for the Diagnosis of Polycystic Ovary Syndrome in Adolescents

Ergenlerde Polikistik Over Sendromu Tanısı için Yeni Biyobelirteçlerin Analizi

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Abstract

Introduction: Polycystic ovary syndrome (PCOS) is a common endocrine problem with complex diagnosis in adolescents. Therefore, it is important to identify reliable biomarkers that can be used in the diagnosis of PCOS in adolescents.
To investigate the diagnostic value of anti-Müllerian hormone (AMH) and inhibin-A (INH-A) and insulin-like peptide-3 (INSL3) in adolescents with PCOS, and to explain the relationship between these hormones and the clinical / laboratory findings of hyperandrogenism.
Materials and Methods: Fifty-five girls aged 15-20 years who were diagnosed with PCOS were included in the present study. The control group consisted of healthy adolescents who had regular menstrual cycles for at least two years and were compatible with the study group according to age and body mass index (BMI). The hormonal profile was assessed in the PCOS and control group. Transabdominal pelvic ultrasonography was performed only in the PCOS group.
Results: AMH and INH-A levels were found to be significantly higher in the PCOS group than control group. While the INSL3 did not correlate with anthropometric or laboratory parameters, AMH level showed a positive correlation with the WC SDS, waist / hip ratio, FAI, LH, fT and INH-A. Moreover, INH-A level showed a positive correlation with WC SDS, LH, LH / FSH ratio, SHBG and AMH. In receiver-operating characteristic analysis, the cut-off value for AMH for the diagnosis of PCOS in adolescents was 5.8 ng / mL that for INH-A was 9.3 pg/mL (the specificity and sensitivity were 86% and 70% and 66% and 82% respectively). When AMH and INH-A were used in combination, the specificity and sensitivity were 74% and 88%, respectively.
Conclusion: INH-A and AMH can be used as new biomarkers for the diagnosis of PCOS in adolescents, while INSL3 has no diagnostic value in this regard.

Keywords
Adolescent, polycystic ovary syndrome, anti-mullerian hormone, inhibin-A, insulin-like peptide-3

Öz

Giriş: Polikistik over sendromu (PKOS), ergenlerde tanısı karmaşık olan yaygın bir endokrin problemdir. Bu nedenle ergenlerde PKOS tanısında kullanılabilecek güvenilir biyobelirteçlerin saptanması önemlidir. Polikistik over sendromlu ergenlerde Anti-Müllerian Hormon (AMH) ve İnhibin-A (INH-A) ve insulin-like peptide-3 (INSL3)'ün tanısal değerini araştırmayı, bu hormonlar ile hiperandrojenizmin klinik / laboratuvar bulguları arasındaki varsa ilişkiyi açıklamayı amaçladık.
Gereç ve Yöntem: Çalışmaya Rotterdam kriterlerine göre Polikistik over sendromu tanısı alan 15-20 yaş arasında 55 kız dahil edildi. Kontrol grubunu en az iki yıl boyunca düzenli adet siklusu olan ve yaş ve vücut kitle indeksine (VKİ) göre çalışma grubu ile uyumlu sağlıklı ergenler oluşturdu. Hormonal profil için açlık kan örnekleri [luteinizan hormon (LH), folikül stimülan hormon (FSH),

Anahtar kelimeler
Ergen, polikistik over sendromu, anti-müllerian hormon, inhibin-A, insulin-like peptide-3

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dehidroepiandrosteron-sülfat (DHEAS), androstenedion (D4-A), total testosteron (tT), serbest testosteron (sT), seks hormonu bağlayıcı globulin (SHBG), AMH, INSL3, INH-A) alındı. Transabdominal pelvik ultrasonografi sadece hasta grubuna yapıldı.
Bulgular: AMH ve INH-A düzeyleri PKOS grubunda kontrol grubuna göre anlamlı olarak yüksek bulundu. INSL3, antropometrik veya laboratuar hiçbir parametreleriyle korelasyon göstermezken, AMH seviyesi bel çevresi (BÇ) standart deviasyon skoru (SDS), bel / kalça oranı, FAI (serbest androjen indeksi), LH, sT ve INH-A ile pozitif bir korelasyon gösterdi. Dahası, INH-A seviyesi BÇ SDS, LH, LH / FSH oranı, SHBG ve AMH ile pozitif korelasyon göstermiştir. ROC analizinde, ergenlerde PCOS tanısında AMH için cut-off değeri 5,8 ng / mL iken INH-A için cut-off değeri 9,3 pg / mL olarak hesaplandı (sırasıyla özgüllük ve duyarlılık %86 ve %70 ve %66 ve %82 idi). AMH ve INH-A birlikte kullanıldığında, özgüllük ve duyarlılık sırasıyla %74 ve %88 idi.
Sonuç: Ergenlerde PKOS tanısında INH-A ve AMH'nın yeni biyobelirteçler olarak kullanılabileceği buna karşı INSL3'ün tanısal değerinin olmadığı gösterilmiştir.

Introduction

Polycystic ovary syndrome (PCOS) is a syndrome, not a disease; it is associated with various symptoms and clinical findings and is one of the most common endocrine disorders in females of reproductive age. While the prevalence of PCOS is around 5%-10% in adult women, increase up to 30% in adolescents. It has been reported that this rate was increased up to 50% in obese adolescents (1,2).

Two of the three criteria specified in the 2003 Rotterdam consensus report must be met for the diagnosis of PCOS; oligo and / or anovulation, clinical and / or laboratory findings of hyperandrogenism, and ultrasonographic polycystic ovaries (3).

When diagnosing PCOS in adolescent girls and young women, other causes of hyperandrogenism should be ruled out. In healthy adolescent girls, the ovaries may normally have a multicystic / polycystic appearance. Moreover anovulation cycles can continue for a while after menarche. Hyperandrogenism findings, such as acne, are more common in adolescents. Moreover, the reference range for androgen levels in adolescents differs from that in adults. Therefore, it is not easy to diagnose PCOS in adolescents (4). Although, numerous studies have been conducted on adult women with PCOS, there are no approved diagnostic criteria and threshold values for hormonal parameters in adolescents with PCOS. Hence, it is important to identify new and effective laboratory markers to confirm the diagnosis of PCOS in adolescents and to manage the treatment process.

The anti-Müllerian hormone (AMH) is an important biomarker currently used for the diagnosis of PCOS in adult women. AMH is produced in the granulosa cells of early developing follicles. The AMH level is too low to be detected at birth; it increases after puberty, possibly as a result of follicular growth, and can be detected until the end of ovarian activity. The AMH level is not affected by fluctuations in other reproductive hormones. Moreover, the level does not change throughout the menstrual cycle. This makes AMH a promising biomarker (5). Various studies have shown that serum AMH levels in adult women with PCOS are higher than those in adult women with normal menstrual cycles. It is thought that this hormone reflects the continuous and off-cycle growth of small ovarian follicles (6,7).

Inhibin-A (INH-A), the bioactive form of inhibin, is synthesized in follicular granulosa cells (8). In healthy women, an increase in the LH level in the middle of the menstrual cycle stimulates the release of INH-A, mostly from the dominant follicles before ovulation. As the follicle grows, the INH-A level increases (8,9). Hence, it can be expected that the INH-A level remains normal or decreases in patients with PCOS with multiple small antral follicles. However, studies on adult women have reported conflicting results. While some studies on adult women with PCOS have reported no change in the INH-A level (10), some have reported an increase (11,12) or a decrease (13,14).

Few studies have investigated AMH and INH-A in the adolescent age group to date.

Another biomarker is insulin-like peptide-3 (INSL3), a member of the insulin family. INSL3 is particularly synthesized by the interna cells of the antral follicles as well as the corpus luteum and ovarian stroma in women. INSL3 levels are reflective of gonadal function (15,16) INSL3 levels, which are similar in prepubertal and postpubertal periods, significantly increase after the onset of puberty (17 Conflicting results have been reported in studies involving adult women. Some studies have reported that INSL3 expression change with follicular development, with higher expression being noted in small antral follicles and lower expression being noted as the follicles become preovulatory. This indicates

that INSL3 levels are negatively correlated with follicular maturation (18). In contrast, Hagen et al. (17) reported that large follicles are better reflective of INSL3 levels than small follicles and that INSL3 is a specific marker for theca cells surrounding larger follicles. Moreover, INSL3 levels have been found to significantly increase in women with PCOS (19,20). INSL3 levels have also been investigated in healthy adolescents (17,21); however, limited studies have investigated the diagnostic value of INSL3 in adolescents with PCOS to date.

In the present study, we aimed to investigate the diagnostic value of AMH, INH-A, and INSL3 in adolescents with PCOS; to explain the relationship between these hormones and clinical/laboratory findings associated with hyperandrogenism; and to determine the age-specific threshold values of these hormones.

Materials and Methods

Fifty-five girls aged 15-20 years who were presented to the pediatric endocrinology outpatient clinic between 2017-2020 with hirsutism and / or menstrual irregularity and were diagnosed with PCOS according to Rotterdam criteria were included in the present study. Adolescents whose had other endocrine disorders (thyroid hormone dysfunction, cushing syndrome, congenital adrenal hyperplasia, etc.), chronic disease, tumor, genetic syndrome and using drug that may affect laboratory findings were not included in the study. The control group consisted of 25 healthy adolescents who had a regular menstrual cycle for at least two years and were compatible with the study group participants in terms of their age and body mass index (BMI). The menstrual cycle interval longer than 45 days was defined as oligomenorrhea and longer than 3 months as amenorrhea. From the data in the files were recorded participants' birth weight, first menstrual age, family history for type 2 DM and PCOS, and their mother's first menstrual age. Written consent was obtained from all parents and participants. Ethics Committee approval was obtained for this study. (Ethics committee decision number 353).

Body weight and height of all participants were measured by the same physician (S.T.) using a digital scale with automatic height measurement (Densi GL 150). BMI was calculated according to the weight (kg) / height (m^2) formula. Waist circumference (WC) was measured at the midpoint between the lower margin of the last palpable rib and the upper part of the iliac crest using an unstretched tape and the hip circumference (HR) was measured from around the widest part of the leg, parallel to the floor (22). The waist/hip ratio (WHR) was calculated. The standard deviation scores (SDS) of these measurements were calculated using national data (23,24). The Ferriman-Gallwey (FG) scoring method was used to define clinical hyperandrogenism (25). In the early morning, fasting blood samples were collected for assessing glucose, insulin, LH, FSH, DHEA-S, D4-A, total testosterone (tT), free testosterone (fT), SHBG, AMH and inhibin-A. Basal 17-hydroxyprogesterone (17-OHP) and cortisol were measured to exclude an adrenal enzyme defect, free T4 (fT4) and thyroid stimulating hormone (TSH) were measured to exclude a thyroid hormone defect and prolactin were measured to exclude intracranial pathologies. The free androgen index (FAI) was calculated using this formula; 100 x (tT / SHBG). Serum LH, FSH, DHEA-S, total T and cortisol concentrations were measured using an electrochemiluminescence (ECL) method (Cobas 6000, Roche). Free testosterone and 17OHP levels were measured using the radio immunoassay (RIA) method. SHBG levels were determined with the chemiluminescent microparticle immunoassay (CMIA) method. Serum INH-A, AMH and INSL3 levels were measured using the enzyme linked immunosorbent test (ELISA) method. Transabdominal Pelvic ultrasonography (USG) examination was performed by the same experienced pediatric radiologist who blinded to clinical and laboratory findings of patients. Pelvic US was performed on the same day as hormonal tests and only in the PCOS group. Three dimensions of the uterus (total uterine length, anteroposterior (AP) and transverse diameters of the corpus), endometrial thickness and three dimensions of each ovary (longitudinal, transverse and AP diameters) were measured by ultrasound. Uterus and ovarian volumes were calculated according to the formula=longitudinal diameter x AP diameter x transverse diameter x 0.52. Each ovary was defined as polycystic, when it contained 12 or more follicles with a diameter of 2-9 mm (26).

Statistical Analysis

SPSS 22.0 package program was used for all statistical analyzes. Results are given as mean±SD

(standart deviation) or median [minimum-maximum]. Chi-square test for categorical variables, independent sample t-test for continuous variables in independent groups, and Pearson correlation analysis for the relationship between variables were used. Mann Whitney U test was used for continuous variables that did not show normal distribution. AMH and INH-A were analyzed using the Receiver Operating Characteristics (ROC) curve analysis and their diagnostic values (sensitivity and specificity) were calculated. When the type-1 error level in the area under the curve (AUC) evaluation was below 5%, the diagnostic value of the test was considered statistically significant. The net sensitivity and specificity rates of the combined use of AMH and INH-A in the diagnosis of PCOS were calculated. A p-value of <0.05 was considered statistically significant.

Results

In the PCOS group, 58% (n=32) of the participants had hirsutism and 85% had oligomenorrhea / amenorrhea, 31% (n=17) had acne, 1.8% (n=1) had alopecia, 43% (n=24) had acanthosis nigricans. Amenorrhea was found in 27% (n=15), and oligomenorrhea was found in 58% (n=32) of the patients with menstrual irregularity. 14.5% (n=8) of the cases with PCOS had normal menstrual cycle. 34.5% (n=19) of the participants had a family history for PCOS and 58.2% (n=32) had a family history for type 2 diabetes mellitus. The distribution of individuals in terms of BMI SDS, mean age, first menarche age and birth weight was similar in the both groups (p> 0.05).

The waist circumference standard deviation score (WC SDS) and waist / hip ratio were significantly higher in the PCOS group than in the control group (p=0.001). Anthropometric measurements in the PCOS and control groups are shown in Table 1. Moreover, the LH level, LH / FSH ratio, tT level, fT level, DHEAS level and free androgen index (FAI) were significantly higher in the PCOS group than in the control group (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, respectively). The SHBG level was lower in the PCOS group than in the control group (p< 0.001) while INSL3 levels were similar between the two groups (p=0.174). AMH and INH-A levels were found to be significantly higher in the PCOS group than in the control group (p<0.001, p=0.009, respectively). The hormone levels in the PCOS and control groups are presented in Table 2.

The FG score showed a positive correlation with the waist / hip ratio, AMH level and FAI (r=0.428, p=0.001, r=0.268, p=0.016; r=0.479, p=0.001, respectively). While the INSL3 level did not correlate with anthropometric or laboratory parameters, the AMH level showed a positive correlation with the WC SDS, waist / hip ratio, FAI, LH level, fT level and INH-A level (r=0.331, p=0.003; r=0.329, p=0.038; r=0.331, p=0.003; r=0.255, p=0.032; r=0.309, p=0.022 and r=0.347, p=0.009, respectively). Moreover, the INH-A level showed a positive correlation with the WC SDS, LH level, LH / FSH ratio, SHBG level and AMH level (r=0.397, p=0.001, r=0.269, p=0.047; r=0.421, p=0.002; r=0.339, p=0.002 and r=0.347, p=0.009, respectively). FAI level correlated positively with FG score, WC SDS, waist / hip ratio and AMH

Table 1. Comparison of clinical and anthropometric characteristics in PCOS and control groups

	PCOS group n=55	Control group n=25	p
Age (years)	15.54±2.37	15.88±1.34	0.771
Menarcheal age (median years)	12 (11-14)	12 (10.5-14)	0.629
Birth weight (gr)	2932±550	2962±539	0.641
BMI SDS	1.44±0.89	1.34±0.67	0.458
WC SDS	2.90±1.66	1.72±0.67	**0.001**
WHR	0.81±0.06	0.53±0.09	**0.001**
FG score	15.98±9.48 (4-32)	6.44±3.29 (4-16)	**0.001**
Familial history of PCOS (%)	%34.5 (n=19)	%16 (n=4)	**<0.023**
Family history of Diabetes (%)	%58.2 (n=32)	%24 (n=6)	**<0.012**

PCOS: Polycystic ovary syndrome, SDS: Standard deviation score, BMI: Body mass index, WC: Waist circumference, WHR: Waist to hip ratio, FG: Ferriman-Gallwey

Table 2. Comparison of laboratory findings in PCOS and control groups

	PCOS group n=55	Control group n=30	p
FSH (mIU/mL)	5.07±1.53	5.24±2.17	0.689
LH (mIU/mL)	9.55±4.99 (2-23)	4.58±1.41	**<0.001**
LH/FSH oranı	2.40±1.52 (0.37-8.7)	1.13±0.68	**<0.001**
tT (ng/mL)	1.55 ±0.6 (0.3-3.6)	0.43±0.21 (0.01-1.2)	**<0.001**
fT (pg/mL)	2.61±1.53 (0.89-6.1)	0.78±0.66 (0.1-2.4)	**<0.001**
DHEA-S (μg/dL)	364±135.7	172±48.3	**<0.001**
FAI	14.38 (2.12-30.65	2.43 (0.08-11.93)	**<0.001**
17-OHP (ng/mL)	1.64±0.72	not measured	-
Androstenedion (ng/mL)	4.10 (0.91-9.5)	not measured	-
SHBG (ng/dL)	9.69 (3.65-35.5)	18.74 (6.8-34.7)	**<0.001**
INH- A (pg/mL)	27.55 (2.3-213)	6.64 (1.2- 21.4)	**0.009**
AMH (ng/mL)	10.12±7.58	4.50 ±1.71	**0.001**
INSL3 (pg/mL)	1197±310.5	1286±276.8	0.174

PCOS: Polycystic ovary syndrome, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, tT: Total testosterone, fT: Free testosterone, FAI: Free androgen index, DHEA-S: Dehydroepiandrosterone sulfate, SHBG: Sex hormone binding globulin, 17OHP: 17-hydroxyprogesterone, AMH: Anti-Müllerian hormone, INH-A: Inhibin-A, INSL3: Insulin-like peptide-3

AMH ROC curve

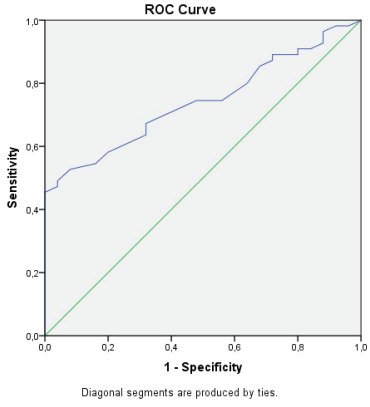

Figure 1. Receiver operating characteristics (ROC) curves of anti-Müllerian hormone (left)

INH-A ROC curve

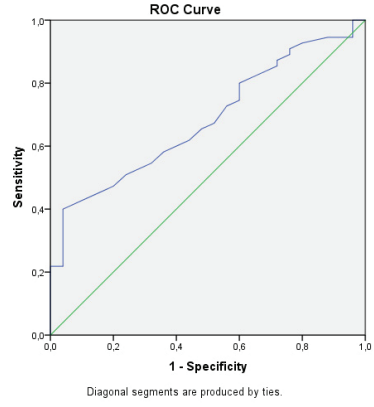

Figure 2. Receiver operating characteristics (ROC) curves of inhibin-A (right)

level (r=0.479, p<0.001; r=0.248, p<0.027; r=0.633 p<0.001; r=0.331, p=0.003, respectively).

In receiver-operating characteristic (ROC) analysis was performed to determine the ability of AMH and INH-A to distinguish between adolescents with PCOS and healthy adolescents (Figure 1,2). The cut-off value for AMH for the diagnosis of PCOS in adolescents was 5.8 ng / mL, while that for INH-A was 9.3 pg/ mL. Based on these cut-off values, the specificity and sensitivity of AMH in the diagnosis of PCOS were 86% and 70%, respectively, while the specificity and sensitivity of INH-A were 66% and 82%, respectively. When AMH and INH-A were used used together, the specificity and sensitivity were 74% and 88%, respectively.

Discussion

To date, very few studies have investigated the diagnostic value of INH-A, INSL3, and AMH in adolescents with PCOS.

Increased WC SDS and WHR are important findings indicative of visceral adiposity. Visceral adiposity causes hyperandrogenemia through insulin resistance. A positive correlation has been reported between the FAI and WC SDS (27,28). In the present study, the WC SDS and WHR were found to be higher in the PCOS group than in the control group. In addition, a positive correlation was noted between the WC SDS and WHR (indicative of visceral adiposity) and the FAI, FG score, AMH level, and INH-A level.

In many studies, AMH levels have been found to increase in patients with PCOS (27,29-31). However, some studies have reported that the increase in AMH levels is not significant (32).

In the present study, AMH levels were found to be significantly higher in the PCOS group than in the control group. Studies involving adolescents with PCOS reported that for AMH, cut-off values of 6.6 ng/mL, 6.1 ng/mL and 7.2 ng/mL have high sensitivity and specificity (27,33,34). However, in another study, ≥7.03 ng/mL AMH showed only 50% specificity and 70.8% sensitivity in the diagnosis of PCOS in adolescents (35). In our study, a cut-off value of 5.8 ng/mL for AMH was found to have 86% specificity and 70% sensitivity in the diagnosis of PCOS.

Regarding the relationship between AMH and other androgens, some studies have revealed that the AMH level correlates with the testosterone level, while some have revealed that the AMH level correlates with the D4-A level and FAI (36,37). In their study involving adolescents with PCOS, Yetim et al. (27) reported that the AMH level is significantly associated with DHEAS, fT, and D4-A levels. In the present study, the AMH level was found to be significantly correlated with the WC SDS, WHR, FG score, fT level, FAI, and INH-A level.

Many studies have reported that INSL3 levels significantly increase in adult women with PCOS (19,20,38). INSL3 levels have also been investigated in healthy adolescents (17,21). To date, very few studies have investigated the diagnostic value of INSL3 in adolescents with PCOS (27). In the present study, INSL3 levels did not correlate with any anthropometric or laboratory parameter in adolescents with PCOS. In a study conducted on 53 adolescents with PCOS, no significant difference was reported between the PCOS and control groups, in accordance with the present findings. In the same study, a weak positive correlation was noted between INSL3 and testosterone levels and a negative correlation was noted between INSL3 and INH-A levels (27).

As the INH-A level is associated with an increase in the LH level and the LH level and LH/FSH ratio increase independently of the cycle in patients with PCOS, the INH-A level is also expected to increase in these patients. However, studies conducted on adult patients with PCOS have reported contradictory results. While some studies have reported no change in the INH-A level (9,10), some have reported an increase (11,12) or a decrease (13,14).

Very few studies have been conducted on INH-A in adolescents with PCOS. Yetim et al. (27) found that the INH-A level significantly increased and its sensitivity was high in adolescents with PCOS. In the present study, the INH-A level was significantly higher in the PCOS group than in the control group (p=0.009).

A study on the relationship of INH-A with other androgens in adolescents with PCOS reported that the INH-A level is positively correlated with the WC SDS, LH level, LH/FSH ratio, SHBG level, and DHEAS level (26). Another study reported a positive correlation between the INH-A level and the LH level and LH/FSH ratio in adults (10). In the present study, the INH-A level was found to be positively correlated with the WC SDS, LH/FSH ratio, and AMH level.

To date, only one study has reported the cut-off value for INH-A in adolescents with PCOS. That study revealed that a cut-off value of 12.8 pg/mL for INH-A has high sensitivity and specificity (86.8% and 69.2%, respectively) (27). In our study a cut-off value of 9.3 pg/mL for INH-A was found to have 82% sensitivity and 66% specificity in the diagnosis of PCOS.

The similarity of age, BMI, and menarche age in the PCOS and control groups is the strength of the present study, as it is important to select compatible groups to accurately evaluate the effects of these parameters on hormone levels.

Study Limitations

One of the limitations of our study was the fact that number of cases were low in the patient and control groups. However, the similarity of age, BMI SDS values and menarche age in both groups, partially eliminates this limitation.

Conclusions

INH-A can be used as a new biomarker in addition to AMH for the diagnosis of PCOS in adolescents. We believe that the cut-off values for AMH and INH-A, which have high sensitivity and specificity, should also be taken notice. Given the insufficient number of studies, larger studies are needed to identify biomarkers and thresholds that can help in the diagnosis of PCOS in adolescents.

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Ethics

Ethics Committee Approval: The Ethics Committee of Diyarbakır Gazi Yasargil Training and Research Hospital (no: 353).

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