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The Relationship Between Anti-Mullerian Hormone and Androgens in Healthy Women Without Hyperandrogenemia

Hiperandrojenemisi Olmayan Sağlıklı Kadınlarda Anti-Müllerian Hormon ile Androjenlerin İlişkisi

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ABSTRACT

Aim: *To determine the relationship between androgens and Anti-Mullerian Hormone in healthy women without hyperandrogenemia.*
Methods: *A total of 1300 patients aged 16-43 whose Anti-Mullerian Hormone and androgen profiles were analysed for a period of three years in a university hospital were included in the study. Socio-demographic features, clinical and sonographic findings, serum prolactin, luteinizing hormone, total and free testosterone, estradiol, thyroid stimulating hormone, follicle stimulating hormone, 17-hydroxy progesterone, dehydroepiandrosterone and Anti-Mullerian Hormone levels were recorded. Patients were selected according to inclusion and exclusion criteria and the study was completed with 337 patients.*
Results: *The mean age of the patients was 28.82±5.24 years and body mass index was 25.49±4.37 kg/m². Mean levels of follicle stimulating hormone, luteinizing hormone and estradiol were 6.82±4.71 mIU/mL, 5.59±3.78 mIU/mL and 40.77±36.28 pg/mL, respectively. When the androgen profiles were evaluated, the mean total testosterone, free testosterone, 17-hydroxy progesterone, androstenedione and dehydroepiandrosterone levels were detected as 47.36±33.09 pg/mL, 2.61±2.54 pg/mL, 0.65±0.20 ng/mL, 2.43±1.25 ng/mL and 244.45±115.87 mcg/dL, respectively. The mean Anti-Mullerian hormone levels were 4.43±4.70 ng/mL. A significant independent relationship was found between Anti-Mullerian hormone and luteinizing hormone (p=0.009), follicle stimulating hormone (p=0.001), androstenedione (p=0.050), dehydroepiandrosterone (p=0.034) and body mass index (p=0.021). Anti-Mullerian hormone levels were affected by follicle stimulating hormone and body mass index negatively; while luteinizing hormone, androstenedione and dehydroepiandrosterone affect its levels positively.*
Conclusion: *According to the results of this study, even in patients who have not hyperandrogenemia or not diagnosed as late-onset congenital adrenal hyperplasia or polycystic ovary syndrome, androstenedione (a precursor of testosterone) and dehydroepiandrosterone may increase Anti-Mullerian hormone levels.*

Keywords: *Androgen, androstenedione, Anti-Mullerian Hormone, dehydroepiandrosterone, testosterone*

ÖZ

Amaç: *Bu çalışmada, sağlıklı kadınlarda Anti-Müllerian hormon ile androjenlerin ilişkisini belirlemeyi amaçladık.*
Yöntem: *Çalışmamıza 16-43 aralığındaki, üç yıllık zaman dilimi içerisinde üniversite hastanemizde anti-müllerian hormon ve androjen profili tetkiki yapılmış 1300 hasta alındı. Hastaların dosyalarından hastaların demografik verileri, klinik ve sonografik bulguları, serum prolaktin, luteinizan hormon, total ve serbest testosteron, östradiol, tiroid stimulan hormon, folikül stimulan hormon, 17-hidroksi progesteron, dihidroepiandrosteron ve Anti-Müllerian hormon seviyeleri kaydedildi. Dahil edilme ve dışlanma kriterleri sonucunda yapılan seçimle çalışmaya 337 hasta ile devam edildi.*
Bulgular: *Çalışmaya alınan hastaların yaş ortalaması 28,82±5,24 yıl ve vücut kitle indekslerinin ortalaması 25,49±4,37 kg/m² idi. Serum folikül stimulan hormon ortalaması 6,82±4,71 mIU/mL, serum luteinizan hormon ortalaması 5,59±3,78 mIU/mL, serum östradiol ortalaması 40,77±36,28 pg/mL olarak bulundu. Hastaların androjen profiline bakıldığında total testosteron değerlerinin ortalaması 47,36±33,09 pg/mL, serbest testosteronun ortalaması 2,61±2,54 pg/mL, 17-hidroksi progesteronun ortalaması 0,65±0,20 ng/mL, androstenedionun ortalaması 2,43±1,25 ng/mL ve dihidroepiandrosteronun ortalaması 244,45±115,87 mcg/dL olarak hesaplandı. Anti-Müllerian hormon değeri ortalaması 4,43±4,70 ng/mL olarak bulundu. Yapılan istatistiksel analiz sonucunda luteinizan hormon (p=0,009), folikül stimulan hormon (p=0,001), androstenedion (p=0,050), dihidroepiandrosteron (p=0,034) ve vücut kitle indeksinin (p=0,021) Anti-Müllerian hormon ile anlamlı ilişki gösterdiği saptandı. Bunlardan folikül stimulan hormon ve vücut kitle indeksinin Anti-Müllerian hormon üzerine azaltıcı; luteinizan hormon, androstenedion ve dihidroepiandrosteronun ise arttırıcı etkisi olduğu görüldü.*
Sonuç: *Bu çalışma sonuçlarına göre hiperandrojenemi, polikistik over sendromu ve geç başlangıçlı konjenital adrenal hiperplazisi olmayan hasta grubunda bile androstenedionun (testosteron öncülü) ve dihidroepiandrosteronun Anti-Müllerian hormonu arttırıcı etkisi olabileceği sonucuna varıldı.*

Anahtar kelimeler: *Androjenler, androstenedion, Anti-Müllerian hormon, dihidroepiandrosteron, testosteron*

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INTRODUCTION

Anti-Mullerian Hormone (AMH), a 140 kDa glycoprotein belonging to transforming growth factor-beta family, is mainly secreted from the granulosa cells starting from 25th gestational week until menopause[1]. While expression of AMH reaches to its highest values during preantral and small antral growing follicle stages it decreases following the determination of the dominant follicle. AMH prevents the exhaustion of premature oocytes through displaying inhibitory effects on early follicular recruitment[2]. Furthermore, at molecular level AMH has an inhibitory effect on Follicle Stimulating Hormone (FSH) induced aromatase expression that leads to decreased estradiol (E2) levels. Also, it inhibits cyclic follicular recruitment via reducing the sensitivity of follicles to FSH[3]. So, AMH can be obviously stated as a regulator of follicular growth and development of FSH sensitivity.

Measurement of serum AMH level has been used as a sensitive marker of ovarian reserve for several years[4,5]. Another advantage is that its serum concentration is quite stable throughout the cycle and the variation in between cycles is very limited[6]. Although the data is not consistent, currently it is known that AMH secretion is affected by several factors and androgens are being discussed from this point of view for several years.

The relationship between androgens, follicular growth and AMH has been investigated in several studies most of which are mainly focusing on polycystic ovary syndrome (PCOS)[7-10].

Previous studies have demonstrated that AMH levels are higher in PCOS patients and correlate with serum androgen levels[4]. Furthermore, the mechanism of regulation of folliculogenesis in patients with PCOS is still under debate. To elucidate this, several studies have been conducted and a relationship between AMH and insulin resistance and between free androgen index and antral follicles were shown[11,12]. According to the currently existing data it is plausible to infer that the androgens are both involved in development of follicles and in dysregulated folliculogenesis of PCOS. However, data about the relationship of AMH and androgens in women without hyperandrogenemia is quite limited.

In this study we assessed the relationship between AMH and androgens in women without hyperandrogenemia.

MATERIAL and METHODS

Study Participants

A total of 1300 patients aged 16-43 who had AMH and androgen profile analysis for any reason in three years period in a university hospital gynecology department were recruited. The study had a retrospective design and it was conducted in a single center. The demographic features, clinical findings and laboratory parameters of patients such as age, gravida, parity, abortion, weight, height, presence of alopecia, hirsutism, seborrhea and acne, length and frequency of menstrual cycle and the amount of menstrual bleeding were obtained from hospital records. Body mass index (BMI) was calculated according to the formula weight (kilogram)/height (m^2). The ultrasonographic findings about the ovaries including polycystic appearance was noted in the patient records. The results of the laboratory tests including serum prolactin, total testosterone (tT), free testosterone (fT), androstenedione, dehydroepiandrosterone (DHEA), 17-hydroxy progesterone (17-OHP), FSH, luteinizing hormone (LH), E2, thyroid stimulating hormone (TSH), insulin, glucose, AMH levels and adrenocorticotropic hormone (ACTH) stimulation test were recorded.

Patients who were diagnosed as PCOS according to the Rotterdam criteria and Late-onset Congenital Adrenal Hyperplasia (LOCAH) according to ACTH stimulation test, patients with any thyroid diseases (TSH >5 mIU/L), hyperprolactinemia (prolactin >30 mIU/mL),increased serum FSH and LH levels (>30 mIU/mL), Cushing Syndrome, any systemic diseases such as diabetes mellitus, hypertension, chronic liver or kidney diseases, auto-immune diseases, patients who had used any hormonal contraceptives, gesta-

gens, corticosteroids, anti-androgenic drugs or those who underwent ovulation induction or controlled ovarian hyperstimulation were excluded from the study .

According to the above- mentioned exclusion criteria 337 out of 1300 patients were included in the study. This study was conducted in accordance with Declaration of Helsinki and it was approved by the local ethic committee (Istanbul University Cerrahpasa Medical Faculty Ethic Committee for Human Studies, 04.04.2014, 2014/8892). All patients provided written informed consent, which was a prerequisite for enrollment.

Biochemical Analysis

Blood samples for measurement of hormone concentrations, on day 3-5 of a spontaneous menstrual cycle which refers to early follicular phase or after a withdrawal bleeding and any day of menstrual cycle for measurement of circulating serum AMH concentrations were obtained from antecubital vein following an eight hour fasting period. On the same day, a transvaginal ultrasonographic examination was performed using a 6 MHz transducer (PVM-651VT, Nemio 20, Toshiba, Japan) to determine the count of early antral follicles with a diameter of 2-9 mm and if present polycystic appearence of the ovaries was recorded.

The blood samples for measurement of circulating AMH concentrations were collected in a lithium containing heparin tube. Plasma was separated within 2 hours after venipuncture, frozen in aliquots at -80°C until thawed and assayed in batches. AMH was measured by ultra sensitive enzyme-linked immunosorbent assay (ELISA) method using a commercially available kit (DSL-10-14400 Active Müllerian Inhibiting Substance/AMH enzyme linked immunosorbent assay kit, Diagnostic Systems Laboratories [DSL], Webster, TX) with GDV device (Beckman Coulter Inc, USA). The sensitivity of the test was 0.006 ng/ml,its interassay coefficient of variation was 6.7% and the intraassay coefficient of variation was 3.3%.

Serum E2, FSH and LH levels were determined using an Immulite 2000 (Diagnostic Products Corp., Los Angeles, CA). Serum 17-OHP, total and free testosterone, DHEA levels were measured with competitive immunoenzymatic colorimetric method (DiaMetraS.r.I. Headquater, Via Garibaldi, 18-20090, Segrate, Milano, Italy). Prolactin, TSH, insulin concentrations and other serum biochemical parameters were studied through enzymatic, photometric and chemiluminescent immunoassay techniques using Roche Hitachi Moduler Analyzer (GmbH mainheim, Germany). HOMA-IR values of the patients were calculated by the formula: [fasting insulin level X fasting glucose level (mg/dl) / 450].

Statistical analysis

SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA) was used for statistical analysis. Kolgomorov-Smirnov test was used to check whether there is a normal distribution of the data. Variables were reported as means and SDs. Backward linear multiple regression analysis was performed to determine the factors associated with AMH which was the dependent variable. A p value <0.05 was considered as statistically significant.

RESULTS

A total of 1300 patients aged 16-43 who had AMH and androgen profiles were analysed. Based on the exclusion criteria 337 out of 1300 patients were included in the study. The mean age of 337 patients who participated in the study was 28.82±5.24 years. Sociodemographic data of the patients including age, gravida, parity, abortion, weight, height and BMI

Table 1. Sociodemographic findings of study participants.

	Mean±Standart Deviation	Range
Age (years)	28.82±5.24	16-43
Gravida (n)	1.02±1.09	0-4
Parity (n)	0.63±0.84	0-4
Abortion (n)	0.34±0.68	0-3
BMI (kg/m²)	25.49±4.37	17.76-40.06

BMI: Body mass index

were given in Table 1. The mean BMI of the patients were 25.49±4.37 kg/m^2.

The laboratory findings of the study participants were displayed in Table 2. The mean serum concentration of FSH, and LH were 6.82±4.71 mIU/mL and 5.59±3.78 mIU/mL, respectively. When the androgen profile of the patients were evaluated, the mean value of total testosterone was 47.36±33.09 pg/mL, free testosterone was 2.61±2.54 pg/mL, androstenedione was 2.43±1.25 ng/mL and DHEA was 244.45±115.87 mcg/dL. We calculated the mean value of the circulating AMH concentrations of the study participants as 4.43±4.70 ng/mL.

Table 2. Laboratory findings of the patients.

	Mean±Standart Deviation	Range
Fasting blood glucose (mg/dL)	81.69±9.92	63-119
Basal insulin (µU/mL)	12.11±8.00	1-64
HOMA-IR	2.45±1.74	0.3-15.08
FSH (mIU/mL)	6.82±4.71	0-27
LH (mIU/mL)	5.59±3.78	0-28
E2 (pg/mL)	40.77±36.28	3.30-401.7
Total testosteron (pg/mL)	47.36±33.09	0.30-174.0
Free testosteron (pg/mL)	2.61±2.54	0-25
17-OHP (ng/mL)	0.65±0.20	0.30-1.0
Androstenedion (ng/mL)	2.43±1.25	0.1-7.70
DHEA(mcg/dL)	244.45±115.87	1.90-912.4
TSH (mIU/L)	1.91±0.92	0.01-5.0
Prolactin (mIU/mL)	14.91±5.82	2.0-30
AMH (ng/mL)	4.43±4.70	0-30

17-OHP: 17-hydroxy progesterone, AMH: Anti-Mullerian Hormone, DHEA: dehydroepiandrosterone, E2: estradiol, FSH: follicle stimulating hormone, LH: luteinizing hormone, TSH: thyroid stimulating hormone.

For the evaluation of independent factors associated with AMH concentrations in the circulation backward linear multiple regression analyses were performed considering fasting blood glucose levels, basal insulin, HOMA-IR, BMI, FSH, LH, E2, tT, fT, 17-OHP, androstenedion, DHEA, TSH and prolactin. Among those LH, FSH, androstenedion, DHEA and BMI were shown to have a significant association with AMH (p=0.009; 0.001; 0.050; 0.034 and 0.021, respectively). FSH and BMI were observed to be inversely related with AMH whereas LH, androstenedion and DHEA had a direct relation with AMH. The factors found to be significantly associated with AMH in regression analysis were shown in Table 3. According to the regression analyses fasting blood glucose levels, basal insulin levels, HOMA-IR, E2, tT, fT, 17-OHP, TSH ve prolactin were not found to affect circulating AMH concentrations (p>0.05).

Table 3. Associations of AMH with BMI and hormone parameters by multivariate linear regression.

	β ± SE	p
LH	0.171 ± 0.864	0.009
FSH	-0.326 ± 0.704	0.000
Androstenedion	0.140 ± 0.273	0.050
DHEA	0.151 ± 0.003	0.034
BMI	-0.142 ± 0.735	0.021

BMI: Body mass index, DHEA:dehydroepiandrosterone, FSH: follicle stimulating hormone, LH: luteinizing hormone.

DISCUSSION

The main findings of this study were as follows; 1) There was an inverse relation between AMH and BMI, 2) AMH was observed to be in a direct relation with serum LH levels, 3) There was an inverse relation between AMH and FSH, 4) AMH was found to be in a direct relation with DHEA and androstenedion, 5) Any relations could not be displayed between testosterone and AMH.

Several factors affect circulating AMH concentrations, one of which is BMI. We find an inverse relation between BMI and AMH. Confirming our results a strong negative correlation was also observed between AMH and BMI in a study including patients with PCOS and in another study including late- reproductive age women[13,14]. It was observed that as the amount of adipose tissue increases the leptin production also increases. Leptin causes a decrease in circulating AMH levels through JAK2/STAT3 pathway by suppressing AMH mRNA expression[15]. This is postulated as the mechanism of action in several studies that are claiming the presence of a negative relationship between BMI and AMH[14].

AMH was observed to be in a direct relation with serum LH levels in this study. This was in accordan-

ce with the results of a study investigating the relationship of AMH with insulin resistance, androgens and basal ovarian follicular status including FSH, LH and antral follicle counts (AFCs) both in PCOS and non-PCOS individuals[13]. Also, the results of the study evaluating the association of AMH with clinical and biochemical markers of PCOS yielded that LH and AMH had a direct correlation[16]. On the other hand, Bungum et al.[17] searched this relation among women who are regularly menstruating and even their results confirmed this positive correlation. Considering the apparent relationship of insulin resistance, LH and hyperandrogemia, the effect of both insulin and LH on AMH secretion or the increased secretion of ovarian androgens due to the LH stimulation may provide an explanation for the aforementioned observations[18].

Another factor found to be in relation with AMH is FSH and we found an inverse relation between the two parameters. In previous studies, it was demonstrated that as FSH increased AMH decreased in non-PCOS group while they were positively correlated in PCOS group[13]. Pigny et al.[4] and Skalba et al.[19] published results that were similar to our findings. AMH which is primarily produced by pre-antral and small antral follicles of granulosa cells counteracts the growth promoting effects of FSH on granulosa cells. This is a mechanism necessary for prompt emergence of folliculogenesis and also for the prevention of premature differentiation of granulosa cells. Therefore, it is possible to conclude that as AMH decreases the responsiveness of growing follicles to FSH also decreases[13,20].

In the present study one of the issues we searched was the relationship of androgens with AMH among healthy women without hyperandrogenemia. According to our results AMH was found to be in a direct relation with DHEA and androstenedione. On the other hand, we could not display any relations between testosterone and AMH. The relationship of AMH with defective folliculogenesis in PCOS patients and also the correlation between circulating AMH concentrations and insulin resistance and androgens has been known for a while[13]. This correlation has been also observed up to now in several studies in women who were not hyperandrogenemic. But, how to interpret this data and clarify the importance of AMH-androgen-FSH relation in normal folliculogenesis is still a matter of debate[21].

The distribution of androgen receptors in ovaries differs in regard of the cell type and stage of follicular growth. Makita et al.[22] showed that androgens together with E2, enhanced growth of oocytes in bovine and promoted acquisition of meiotic competence in in vitro cultures. Like androgens, AMH is also known to be secreted mostly by small pre-antral and antral follicle granulosa cells. Apparently it participates in the process of gonadotropin-independent follicular development. One of the two main functions of AMH is that it regulates the initial follicular recruitment negatively and the other is that it inhibits FSH-dependent cyclic recruitment[21]. The final effect of AMH is to protect the primordial follicle pool[23,24].

In the present study, a direct correlation (although not very strong) between, AMH and DHEA was shown. In the study by Kevenaar et al.[25] investigated PCOS patients, and observed that AMH decreased the aromatase activity which was induced by FSH in the early antral follicle granulosa cells and as a result, increased androgen levels were determined. DHEA is thought to be responsible of increasing antral follicle population. Tandemly, circulating AMH concentration increases as the number of antral follicles increase. Also, increased intra-follicular production of AMH due to DHEA stimulation contributes to the increased concentration of AMH in circulation[26].

According to our results while AMH was found to be in association with androstenedion but without any correlation between AMH and testosterone. Nardo et al.[13] demonstrated a direct relationship between testosterone and AMH levels both in PCOS and non-PCOS groups. But the association of AMH and testosterone was not stronger in either PCOS or non-PCOS groups. Likewise, there are studies reporting similar correlations between testosterone and AMH in both

women with PCOS or in healthy controls[27,28]. However, there are other studies which demonstrated a direct relation between AMH and testosterone merely in PCOS group[4]. It was stated that testosterone induces follicular growth through FSH and IGF-I[29]. The level of AMH in circulation was demonstrated to be independently related with ovarian androgenic functions and development of polycystic ovaries[30].

As a limitation of our study it is possible to state that we evaluated the circulating hormone concentrations and AMH, which may not mirror the intraovarian hormonal status. So, studies particularly focusing on the hormonal milieu in the ovarian tissue itself are required to make accurate conclusions about the effects of AMH and androgens on normal or defective folliculogenesis.

In conclusion, in patients who have not hyperandrogenemia or not diagnosed as PCOS or LOCAH, androstenedione and DHEA were found to increase the AMH levels. It is obvious that there is a continuous interaction between FSH, AMH and androgens throughout the menstrual cycle. Their relation with each other changes according to the stage of follicular development but they seem to be always in close correlation. Their effect on each other is crucial not for only follicular recruitment and for the selection of leading follicle but also for ovarian aging. We believe that our findings may provide a contribution to the literature about the comprehension of folliculogenesis.

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