ANTIMÜLLERIAN HORMONE AS INDICATOR OF OVARIAN DYSFUNCTION

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Abstract

Aim. The purpose of this study was to examine various hormonal, biochemical and environmental factors (i.e., smoking and alcohol intake) and to investigate their possible correlation to the development of polycystic ovary syndrome (PCOS). The main objective was to evaluate the associations between hormonal profile and the antimüllerian hormone (AMH) levels in PCOS patients and their relation to environmental factors.

Patients and Methods. In two gynecological clinics, 38 women with PCOS (defined according to the Rotterdam criteria) were enrolled and observed in relation to AMH, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), Δ 4-androstendione (Δ 4-A), dehydroepiandrosterone sulfate (DHEA-S) and glucose plasma concentrations. Obesity, smoking and alcohol exposure were also studied.

Results. AMH, T, Δ 4-A, DHEA-S, LH and FSH were increased in 76.3%, 50%, 31.8%, 23.7%, 21% and 18.4% of the patients, respectively. The LH/FSH ratio and glucose concentrations increased abnormally in 18.4% and 15.8% of the patients, respectively. AMH and T levels were both increased in 47.4% of the patients whereas both AMH and LH levels increased in 21% of the patients. Smoking, alcohol intake, obesity and glucose concentrations were not associated with AMH concentrations. On the contrary, high levels of T and LH were linked to higher levels of AMH. FSH concentrations were not increased in these patients.

Conclusion. AMH is an important hormonal parameter for the diagnosis of PCOS. Larger clinical controlled studies are necessary in an effort to further investigate the inclusion of AMH measurement in the diagnostic criteria of PCOS.

Key words: Polycystic ovary syndrome (PCOS), Stein-Leventhal syndrome, Antimüllerian hormone (AMH), Androgens, Testosterone, luteinizing hormone (LH).

INTRODUCTION

Anti-Müllerian hormone (AMH), also known by various other names (including Müllerian Inhibiting Substance [MIS]) is a dimeric glycoprotein and a member of the transforming growth factor-beta (TGF- β) superfamily. AMH was isolated in 1984. It is primarily known for its role in male sexual differentiation, mainly produced by the Sertoli cells of the tests (1). Its principal activity is the involution of Müller's ducts (uterus, Fallopian tubes and the upper third of the vagina) in male fetuses at 8 to 10 weeks of fetal life. In females, the hormone is produced by one cell type, the granulosa ovarian cells (2). AMH is expressed in granulosa cells at all stages of folliculogenesis. Its highest presence is observed in pre-antral and in small antral follicles. AMH decreases the responsiveness of growing follicles to the follicle stimulating hormone (FSH) and, after the selection of follicles, the level of AMH decreases (3). In murine studies, AMH decreases the number of luteinizing hormone (LH) receptors in oocytes (a process mediated by the FSH) (4) and inhibits the first meiotic division of these cells. Also, AMH has been positively correlated with testosterone (5-6), Δ 4-androstenedione (7) and LH (8). Thus, its role has been suggested in polycystic ovary syndrome (PCOS) pathophysiology.

In the neonatal period, a transient increase in AMH is observed, followed by a sustained rise during childhood and adolescence. AMH continues to increase after puberty and a maximum concentration is noted at about the age of 24.5, decreasing afterwards progressively until menopause (9). Indeed, there is an independent effect of race/ethnicity in the fall of AMH, depending on the woman's age (10). Factors that determine the presence of AMH are not well

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understood. There is evidence that its secretion is regulated by androgens, germ cells and, probably, FSH. However, there are no indications that estrogens are involved in its regulation.

AMH serum concentration correlates with the number of growing follicles because its secretion is found in all growing follicles (5,7). Furthermore, there is an exceptional correlation between AMH serum concentrations and the number of antral follicles (antral follicles count, AFC) shown during an ultrasound (11). Actually, the measurement of AMH in serum is more sensitive and specific than AFC because it is linked to the preantral and small antral follicles (<2 mm) as well, which are poorly visible via the ultrasound exam.

The interpretation of AMH concentrations presents some difficulties because this test is not used as a routine process and the limits of normality have not been well defined. In fact, all modern commercial tests do not provide equivalent results. Below, there is a proposed value range for the interpretation of AMH levels (12), based on the available literature regarding fertility. Serum AMH levels may be influenced by many factors. Obesity has been often associated with significantly lower serum AMH levels, a result not found in all studies (13-15). There is also a controversy regarding the effect of hormonal contraception on its levels. Some authors suggest that combined contraception does not affect its serum levels, while others have recently reported serum levels are reduced by 29 to 50% which can be explained by the suppression of the secretion of gonadotropins (16,17). In addition, AMH is an indicator of follicular reserve, predicting menopause.

PCOS is the most common cause of chronic anovulation accompanied by oligomenorrhea or amenorrhea and it affects 5 to 10% of the female population (18). Moreover, PCOS is the most frequent cause of hyperandrogenism in young women and this is related to their cardiovascular risk (19). The excessive secretion of AMH by non-growing follicles in the ovary is considered by many authors a major element of the syndrome (20). PCOS can be diagnosed according to the Rotterdam 2003 consensus, requiring at least two of the three following criteria: i) menstrual cycle disorder, ii) clinical or biological hyperandrogenism, iii) excess of follicles with antrum shown in the ultrasound exam with ≥ 12 follicles in diameter from 2 to 9 mm per ovary and/or ovarian size ≥ 10 ml (21).

The Rotterdam criteria is the most widely accepted form of diagnosing PCOS, the National Institutes of Health (NIH) criteria as well as those of the Androgen Excess Society (AES) (22) are also used in clinical practice (Table 1). It appears that the classification of PCOS in four categories, depending on different combinations of the three criteria (chronic anovulation, hyperandrogenism, polycystic ovaries), is the most accurate (Table 2) (23).

The deleterious consequences of the syndrome include the future onset of type II diabetes, complications of obesity (when obesity is present), metabolic syndrome and cardiovascular diseases (24), as well as an increased risk of endometrial and breast cancer. Additionally, hirsutism is considered the predominant dermatological manifestation of androgen excess. The following laboratory findings are part of the hormonal profile of PCOS: increased ratio of LH/FSH (> 2), increased or

Table 1. Guidelines for the diagnosis of PCOS

Diagnosis of PCOS	
National Institutes of Health Criteria (2 criteria)	- Hyperandrogenism - Abnormal menstruation
Androgen Excess-PCOS Society Criteria (2 criteria)	- Hyperandrogenism - Ovarian function (oligo-anovulation and/or polycystic ovary morphology) - Exclusion of other hyperandrogenic disorders or ovulatory disorders
Rotterdam Criteria (2 of the 3 criteria)	- Hyperandrogenism - Abnormal menstruation - Polycystic ovaries in ultrasound

Table 2. Different phenotypes (A, B, C, D) of PCOS

4 different phenotypes of PCOS	
Type A	Hyperandrogenism, Chronic Anovulation and Polycystic ovaries
Type B	Hyperandrogenism and Chronic Anovulation
Type C	Hyperandrogenism and Polycystic ovaries
Type D	Chronic Anovulation and Polycystic ovaries

normal LH, normal or reduced FSH, increased total testosterone, increased $\Delta 4$ -androstenedione, increased DHEA-S, increased estrone, normal or increased 17β -estradiol, increased insulin, slightly increased prolactin and reduced SHBG (25).

MATERIAL & METHODS

The aim of this study was to measure various biochemical and hormonal markers, as well as life habits such as smoking and alcohol intake, and to investigate their disrupting contribution in women with PCOS. In particular, the main objective was to investigate the association of biochemical parameters and environmental factors with the increased value of AMH. This was a cross sectional study. Inclusion criteria included reproductive age, and, at least two of the Rotterdam criteria for PCOS diagnosis (abnormal menstrual cycles, chemical or clinical hyperandrogenism and polycystic appearance of the ovaries on ultrasound); 38 women with PCOS were enrolled in the study from 2015 to 2016, after obtaining their informed consent. The sample came from two Greek hospitals, one public and one private. All participants had a spontaneous onset of puberty and normal sexual development. Thyroid dysfunction, hyperprolactinemia, Cushing's syndrome, congenital adrenal hyperplasia, a current or previous pregnancy (within one year), ovarian cancer, autoimmune diseases, tumors, the disease of the central nervous system, the current use of oral contraceptives (or in the previous 6 months) or the use of drugs (which are known to influence the hypothalamic-pituitary-ovarian axis) were considered as exclusion criteria. The average age of the sample population was 32.5 years.

According to Body Mass Index (BMI) the patients were assigned to six categories: women with a BMI $<18.5 \text{ kg/m}^2$ were classified as underweight, women with a BMI: $18.5\text{-}24.9 \text{ kg/m}^2$ were classified as normal, BMI: $25\text{-}29.9 \text{ kg/m}^2$ were classified as overweight; BMI $30\text{-}34.9 \text{ kg/m}^2$ were classified as first degree obese, BMI: $35\text{-}39.9 \text{ kg/m}^2$ as second degree obese and finally, women with a BMI $\geq 40 \text{ kg/m}^2$ were classified as third degree obese (morbid obesity). Blood samples were collected in the morning from all participants in the early follicular phase (day 3 to 5) of spontaneous menstruation, after an overnight fast. In the case of amenorrhea, progestins were prescribed to induce menstruation. In those cases, blood samples were collected 3 to 5 days after the initiation of withdrawal bleeding. In all cases (those with spontaneous

menstruation and those with withdrawal bleeding), levels of AMH, FSH, LH, T, $\Delta 4$ and DHEA-S were measured. The blood samples were centrifuged immediately after collection and subsequently stored at -20°C until assayed. All hormone concentrations were determined using the Electro-Chemiluminescence Immunoassay (ECLIA). AMH levels were evaluated using a competitive immunoassay, an enzyme inhibition technique for the *in vitro* quantitative measurement of AMH (ABIN414951).

A trans-vaginal ultrasound exam, with a 6.5 MHz probe was performed on all the patients in order to diagnose the syndrome. Ultrasound measurements were taken in real time by an experienced physician in each hospital, both in accordance with a standard protocol in order to avoid differences in measurements. Volume was calculated for each ovary by approximating at an elongated ellipsoid: $\pi/6 \times (D1 \times D2 \times D3)$, where D represents the maximum diameter in cross, anterior-posterior and large incision or $(0.5 \times \text{width} \times \text{length} \times \text{thickness})$ (26). The average volume of both ovaries was defined as the ovarian volume.

For the statistical analysis of the study data, the 2007 edition of Microsoft Excel was used. Moreover, an χ^2 test was applied because all the data was categorical. The level of statistical significance was set at $p < 0.05$. As described below, there are no results for the levels of $\Delta 4$ -androstenedione for all participants, but only for 22 of the 38 women who enrolled in this study.

RESULTS

Environmental factors

In this study, we try to observe two of the most important environmental factors, alcohol intake and smoking. Specifically, five of the 38 women (13.2%) consumed >5 glasses of alcohol per week, while 33 (86.8%) did not consume any alcohol. Also, 12 of the 38 women were smokers (31.6%), while 26 (68.4%) were non-smokers. Indeed, 6 of 12 smokers told us that they smoked >15 cigarettes per day. Three of the 38 participants (7.9%) were underweight, 23 (60.6%) had normal weight, 9 (23.7%) were overweight, 1 (2.6%) had first degree obesity, 1 also had second degree obesity (2.6%) and finally 1 (2.6%) had morbid obesity. Altogether, 12 of the 38 women (31.6%) had increased body weight.

Glucose

In this study, we assessed the blood glucose. A blood glucose $>100 \text{ mg/dL}$ was set as a pathological

one. It was found that 6 of 38 women (15.8%) had abnormal basal glucose values.

Gonadotropins

In this study, we also evaluated the FSH and LH sampled in days 3-5 of the menstrual cycle. Values for FSH <10 mIU/mL and for LH <10 mIU/mL were defined as normal. According to the results, 7 of the 38 women (18.4%) had increased levels of FSH, i.e., FSH was ≥ 10 mIU/mL, while 31 (81.6%) had normal FSH, i.e., FSH <10 mIU/mL. Also, 8 of the 38 participants (21%) had increased values of LH, i.e., LH was ≥ 10 mIU/mL, while 30 (79%) had normal levels, i.e., LH <10 mIU/mL.

LH/FSH ratio

It is known that in women with PCOS there is an increased LH/FSH ratio (>2). In this study, it was observed that only 7 of the 38 women who participated (18.4%) showed an increased ratio of LH/FSH (>2).

Androgens

In this study, we assessed the levels of total testosterone (T), of $\Delta 4$ -androstenedione and of dehydroepiandrosterone sulfate (DHEAS). Values for T <0.95 ng/mL were defined as normal. Furthermore, values for $\Delta 4$ -androstenedione <3.5 ng/mL were defined as normal. Moreover, DHEAS values <380 μ g/dL were considered normal. Based on the above normal levels, 19 of 38 women (50%) had normal testosterone levels, while the other 19 (50%) had abnormal levels, i.e., T ≥ 0.95 ng/mL. For $\Delta 4$ -androstenedione, there are results only for 22 of the 38 women: 15 of the 22 (68.2%) had normal levels of $\Delta 4$ -androstenedione, and 7 (31.8%) had increased levels, i.e., $\Delta 4$ -androstenedione ≥ 3.5 ng/mL.

Finally, it appeared that 29 of the 38 women (76.3%) had normal DHEAS levels, while 9 (23.7%) had increased levels, i.e., DHEAS ≥ 380 mg/dL. In 22 of the 38 women for whom we had results for all levels of androgens, we verified whether several androgens simultaneously increased. It was observed that T increased in 11 of the 22 women (50%), $\Delta 4$ -androstenedione increased in 2 of 22 (9%), DHEAS increased in only 1 of 22 women (4.6%) and 3 women out of 22 (13.6%) had no increase in androgen levels. Additionally, 2 of the 22 participants (9%) showed increased levels of T and the $\Delta 4$ -androstenedione, 1 of 22 (4.6%) had increased levels of T and DHEAS, also 1 of the 22 (4.6%) showed increased levels of $\Delta 4$ -androstenedione and DHEAS. Furthermore, only 1 of 22 women (4.6%) had increased levels of all

androgens (T, $\Delta 4$ -androstenedione and DHEAS). Consequently, according to the above results, 19 of the 22 women (86.4%) had at least one of androgens increased. Specifically, it was observed that 14 of 22 women (63.6%) had only T increased. Just 1 of the 22 (4.6%) showed an increase in all androgen levels simultaneously. Therefore, when we checked the levels of androgens in each woman in the sample, it was much more common to find an abnormal level in one of the androgens than to find it in all three.

Antimüllerian Hormone

The Antimüllerian hormone (AMH) was measured in all participants of this study. Taking into account that in women under age 35, AMH values >4.0 ng/mL are considered high and values between 1.5 and 4 ng/mL are considered normal, values for AMH <3 ng/mL were defined as normal in our sample. According to the results, 9 of the 38 women (23.7%) had normal AMH levels, while 29/38 participants (76.3%) had AMH ≥ 3 ng/mL.

Correlation between AMH and women's age

In this study, we searched a correlation between AMH and women's age. Specifically, all women aged 19-25 had AMH ≥ 3 ng/mL. Nine of 11 women aged 26-30 (81.8%) had AMH ≥ 3 ng/mL. Also, 8 of 10 women aged 31-35 (80%) had AMH ≥ 3 ng/mL. Moreover, 6 of 9 women (66.7%) aged 36-40 had AMH ≥ 3 ng/mL. Finally, 2 of 4 (50%) women aged >40 years had AMH ≥ 3 ng/mL. Consequently, based on the above data, there is a decreasing amount of AMH with the increasing age of women (expected, since AMH is used as an indicator of follicular reserve). By dividing the sample into two categories, we observed that 4 of 25 women (16%) aged 19-35 had normal levels of AMH, i.e., AMH <3 ng/mL, while 21 women (84%) had AMH ≥ 3 ng/mL ($p=0.12$). Also, 5 of 13 women (38.5%) aged 36 to >40 had normal AMH levels, i.e., AMH <3 ng/mL, while 8 women (61.5%) had AMH ≥ 3 ng/mL.

Correlation between AMH and smoking

In our study, 3 of 12 smokers (25%) had AMH <3 ng/mL, while 9 of 12 (75%) had increased levels of AMH. Also, 6 of 26 non-smokers (23.1%) had AMH <3 ng/mL, while 20 of 26 non-smokers (76.9%) had increased AMH. In other words, of the 29 women with increased AMH levels, i.e., AMH ≥ 3 ng/mL, 9 were smokers (31%) and 20 were non-smokers (69%). Additionally, of the 9 women who had normal AMH

levels, 3 (33.3%) were smokers and 6 (66.7%) were non-smokers ($p=ns$).

Correlation between AMH and alcohol intake

In our study, from 5 participants consuming alcohol, 4 had increased and 1 had normal AMH levels. Additionally, of the 33 women in the sample that did not consume alcohol, 25 (75.8%) had increased AMH, while 8 of 33 had normal AMH levels. Of the 29 women with increased AMH levels, 4 consumed alcohol, while 25 did not. Only 1 of 9 women who had normal AMH levels consumed alcohol.

Correlation between AMH and BMI

According to the results, 22 of 26 women (84.6%) with normal BMI ($<25 \text{ kg/m}^2$) exhibited elevated levels, $\text{AMH} \geq 3 \text{ ng/mL}$, while 4 of 26 (15.4%) had normal AMH levels. Also, 7 of 12 women (58.3%) with overweight or obesity ($\text{BMI} \geq 25 \text{ kg/m}^2$) exhibited elevated levels of AMH, i.e., $\text{AMH} \geq 3 \text{ ng/mL}$, while 5 of 12 (41.7%) had normal AMH levels, i.e. $\text{AMH} < 3 \text{ ng/mL}$. Of the 29 women with increased AMH levels, 22 (75.9%) had a normal BMI and 7 (24.1%) had an increased BMI. Additionally, of the 9 women who had normal AMH levels, 4 had a normal BMI and 5 (55.6%) had an increased BMI ($p=ns$).

Correlation between AMH and diabetes

In this study, we evaluated the correlation between AMH and diabetes. It was found that 6 of 38 women (15.8%) had increased basal blood glucose ($>100 \text{ mg/dL}$), and these women had also increased $\text{AMH} \geq 3 \text{ ng/mL}$. On the other hand, 32 participants had normal blood glucose levels and 23 / 32 participants (71.9%) had $\text{AMH} \geq 3 \text{ ng/mL}$, while 9 women had $\text{AMH} < 3 \text{ ng/mL}$ ($p=ns$).

Correlation between AMH and gonadotropins

In a sample of 7 women who had abnormal levels of FSH, i.e., $\text{FSH} \geq 10 \text{ mIU/mL}$, 3 women (42.9%) had increased AMH levels, while 4 (57.1%) had normal AMH. Furthermore, in a group of 31 women with normal FSH levels, 26 (83.9%) had increased, while 5 had normal AMH levels.

Moreover, in a group of 29 women with $\text{AMH} \geq 3 \text{ ng/mL}$, 26 (89.7%) had normal levels of FSH, while 3 (10.3%) had high levels of FSH, i.e., $\text{FSH} \geq 10 \text{ mIU/mL}$. From 9 women with normal AMH levels, 5 (55.6%) had normal levels of FSH, and 4 (44.4%) had increased levels of FSH, i.e., $\text{FSH} \geq 10 \text{ mIU/mL}$ ($p=0.02$). Consequently, as expected, we observe

that FSH has a significant relationship with AMH. In a sample of 8 participants who had increased levels of LH, ($\text{LH} \geq 10 \text{ mIU/mL}$), all participants (100%) also had increased levels of AMH, i.e., $\text{AMH} \geq 3 \text{ ng/mL}$. Additionally, from 30 women who had normal LH levels, 21 (70%) had increased AMH, whereas 9 (30%) had normal levels of AMH. In other words, in 29 women with increased AMH levels, i.e. $\text{AMH} \geq 3 \text{ ng/mL}$, 21 (72.4%) had normal levels of LH, i.e., $\text{LH} < 10 \text{ mIU/mL}$, while 8 (27.6%) had abnormal levels of LH, i.e., $\text{LH} \geq 10 \text{ mIU/mL}$. Of 9 women who had normal AMH levels, all 9 (100%) had normal LH levels, i.e., $\text{LH} < 10 \text{ mIU/mL}$ ($p=0.08$, i.e., $p=ns$).

Additionally, we studied the correlation between AMH and the values of FSH and LH. None of the participants had both levels of FSH and LH increased. Of 24 women who had simultaneously reduced levels of FSH and LH, 5 (20.8%) had normal levels of AMH $< 3 \text{ ng/mL}$, while 19 (79.2%) had increased AMH levels $\geq 3 \text{ ng/mL}$. From 6 participants with increased FSH and decreased LH, 4 had AMH $< 3 \text{ ng/mL}$, while 2 had $\text{AMH} \geq 3 \text{ ng/mL}$. Of 8 women with reduced FSH and increased LH, all had increased AMH levels. Altogether, from 29 women with increased AMH levels, none had both levels of FSH and LH increased; 19 of 29 had simultaneously reduced levels of FSH and LH.

Correlation between AMH and LH/FSH ratio

All of 7 women (100%) who had an increased LH/FSH ratio (>2) had increased AMH levels, i.e., $\text{AMH} \geq 3 \text{ ng/mL}$. 22 of 31 women (71%) who had an increased LH/FSH ratio, also had increased AMH levels, whereas 9 (29%) had normal levels of AMH. In other words, 22 of 29 women (75.9%) with increased AMH levels, i.e. $\text{AMH} \geq 3 \text{ ng/mL}$, had normal LH/FSH ratio (≤ 2) and 7 (24.1%) had an increased LH/FSH ratio (>2). All of 9 women (100%) with normal AMH levels, i.e., $\text{AMH} < 3 \text{ ng/mL}$ had a normal LH/FSH ratio (≤ 2) ($p=0.1$, i.e., $p=ns$).

Correlation between AMH and androgens

In this present study, we evaluated the correlation between AMH and androgens. Specifically, we assessed the levels of total testosterone (T), $\Delta 4$ -androstendione and dehydroepiandrosterone (DHEAS) and their correlation to AMH levels. From 19 participants with increased total testosterone levels, i.e., $\text{T} \geq 0.95 \text{ ng/mL}$, 18 (94.7%) had increased AMH levels, i.e., $\text{AMH} \geq 3 \text{ ng/mL}$ and 1 (5.3%) had normal AMH levels, i.e., $\text{AMH} < 3 \text{ ng/mL}$ ($P=0.008$). Also, 11

of 19 women (57.9%) with normal testosterone levels ($T < 0.95$ ng/mL) had increased AMH levels, while 8 (42.1%) had normal AMH levels.

As we already mentioned, there are no results for $\Delta 4$ -androstenedione for all the participants in the study, but for 22 of 38 women who participated in this study. In 7 women who had increased levels of $\Delta 4 \geq 3.5$ ng/mL, we found increased AMH levels. It is noteworthy that from 15 patients who had a normal $\Delta 4$, all 15 (100%) had increased AMH levels.

In other words, none of women from whom we collected results for $\Delta 4$ levels had normal levels of AMH, i.e., $AMH < 3$ ng/mL. Thus, in 22 women with increased AMH levels, i.e., $AMH \geq 3$ ng/mL, 15 (68.2%) had normal $\Delta 4$ levels, i.e., $\Delta 4 < 3.5$ ng/mL and 7 (31.8%) had abnormal $\Delta 4$ levels, i.e., $\Delta 4 \geq 3.5$ ng/mL ($p = 0.63$, i.e. $p = ns$). Finally, we assessed the levels of dehydroepiandrosterone (DHEAS), and found that 8 of 9 women (88.9%) who had increased levels, i.e., $DHEAS \geq 380$ mg/dL, also had increased AMH levels, while only 1 participant (11.1%) had a normal AMH level. Moreover, 21 of 29 participants (72.4%) with normal levels of DHEAS, i.e., $DHEAS < 380$ mg/dL, had increased AMH levels, whereas 8 (27.6%) had normal AMH levels. Therefore, 21 of 29 women (72.4%) with increased AMH levels, i.e., $AMH \geq 3$ ng/mL, had normal levels of DHEAS, i.e., $DHEAS < 380$ mg/dL, while 8 (27.6%) had abnormal DHEAS levels, i.e., $DHEAS \geq 380$ mg/dL. Moreover, 8 of 9 women (88.9%) with normal AMH levels, i.e., $AMH < 3$ ng/mL, had normal levels of DHEAS, i.e., $DHEAS < 380$ mg/dL and 1 (11.1%) had abnormal levels of DHEAS, i.e., $DHEAS \geq 380$ mg/dL ($p = 0.31$, i.e. $p = ns$).

Correlation between AMH, T and LH

In this study, we estimated how many participants showed increased levels of AMH, LH and T, the most significant factors linked to polycystic ovary syndrome. Our findings showed that 5 of 39 participants (12.9%) had increased levels of AMH, LH and T simultaneously. 18 of 38 women (47.4%) had simultaneously increased AMH and T levels, while 8 of 38 (21%) had simultaneously increased AMH and LH levels.

DISCUSSION

Our results show that women who visit gynecological clinics with menstrual disorders due to PCOS and who possibly have the desire of pregnancy are often between the ages of 26-35. Moreover, several

participants are between 36-40 years, while a noteworthy fact is that women aged < 25 years or > 40 years are a minority. Women who drink alcohol (> 5 glasses/week) constitute 13.2% of our sample, confirming the published data on alcohol consumption amongst the female population in Greece that reaches 13.6% (27). Similarly, 31.6% were smokers, a figure comparable to that of the general population in Greece of almost 32% (28). Furthermore, women in this study mainly had a normal BMI. Indeed, overweight patients were three times less than women with a normal BMI. However, this finding is contrary to the available literature, in which women, who have PCOS, frequently have a higher BMI (29). Studies have also supported PCOS in thin women as well (30). In accordance with the classical view, PCOS is associated with hyperglycemia and hyperinsulinism (31). However, in this study, only a few women had abnormally high blood glucose levels.

After the evaluation of the pituitary hormones FSH and LH, it was observed that gonadotropins were increased in very few cases, and were normal in most. Indeed, an increased difference between FSH and LH was not detected, something that was also evident from the results of ratio: LH/FSH. However, classic LH is found to be more increased in PCOS (32).

According to our results, T appears to be an important indicator for PCOS. One in two women showed an increased T value, while $\Delta 4$ and DHEAS were not as high. Consequently, it appears that T is superior as a marker against other androgens, confirming already existing known literature on the topic (33). It is rare for three androgens (T, $\Delta 4$ and DHEAS) or two of three androgens to increase simultaneously whereas only T showed to have increased more.

Subsequently, notable is that AMH was often more increased in contrast with other parameters in the sample. It has been suggested that AMH levels are related to the severity of PCOS (8). Actually, in our study, three in four women appeared to have an increased AMH. In accordance with the literature and our own experience, we propose the following classification for AMH values: High level: > 3.0 ng/mL, Normal level: > 1.0 ng/mL and < 2.9 ng/mL, Minimum normal level: ~ 0.7 - 0.9 ng/mL, Low level: 0.3 - 0.6 ng/mL and Very low level: < 0.3 ng/mL. Additionally, it was observed that AMH decreased as the age of the participants increased which is proof of the decline of ovarian reserves. This finding is in agreement with the literature (34). With regard to smoking and its correlation to AMH, there is no consensus between

the published studies. Some researchers conclude that smoking is associated with reduced levels of AMH (35), which is not true for former smokers (35), while others conclude that smoking does not affect the AMH levels (36). Our findings indicated that smokers did not have a more reduced AMH than non-smokers. Moreover, women who had an increased AMH were more often nonsmokers.

Similarly, alcohol has classically been associated with decreased AMH levels (37).

In contrast, according to our results, women who consumed alcohol did not appear to have a lower AMH, compared with those who did not consume alcohol. Moreover in our series, women who did not consume alcohol showed an increased AMH.

Obese women did not oftentimes show increased AMH levels, while thin and normal weight women, who are the majority of the sample, did not have lower levels of AMH. Furthermore, many of the participants who had increased AMH levels, had normal weight, which confirms findings in the already known literature (38), in which obesity can often be associated with significantly lower AMH levels (15).

From the study focused on the correlation between AMH and diabetes, it was observed that participants who had elevated blood glucose levels always had elevated AMH. Meanwhile, none of the women who had reduced levels of AMH had elevated blood glucose levels, a conclusion which is similar in other studies (24).

The study of the correlation between gonadotropins and AMH showed that AMH is more often increased compared to FSH (76.3% against 18.4%). FSH was not increased in women with high AMH. Furthermore, when AMH was increased, FSH was not as increased. However, when FSH was increased, AMH was not increased. Indeed, in this case, AMH was slightly more reduced. This result is of extreme importance, as a statistically significant relationship between AMH and FSH was found. This conclusion has been demonstrated in other studies (39).

Regarding the correlation between AMH and LH, it was found that AMH is more often increased than LH (76.3% vs. 21%). When AMH was increased, LH did not so often increase. While, unlike FSH, when LH was high AMH was always increased, which is also known from other studies (6, 8).

Additionally, FSH and LH were observed at normal levels. Secondly, LH was increased while FSH was at normal levels. Moreover, women with increased AMH levels had the same frequency of FSH and LH,

which has been observed in other studies (39).

Women who had increased AMH levels often showed a decreased ratio of LH/FSH. Furthermore, none of the women who had normal AMH levels showed an increased ratio of LH/FSH. In fact, there are studies that contest the usefulness of the LH/FSH ratio used to assess PCOS (39). Consequently, AMH seems to be more important than the LH/FSH ratio. It is worth noting that, of the women who had an increased LH/FSH, AMH was increased in all cases. This result coincides with the results of other studies (6).

In the study of the correlation between AMH and T, we found that AMH is more often increased compared to T (76.3% versus 50%). Women, who had an increased T, almost always had an increased AMH. On the other hand, women who had an increased AMH, more frequently presented an increased T. Indeed, according to our sample, the relationship between T and AMH was statistically significant. This finding is also a conclusion of other studies (6).

In the subgroup of $\Delta 4$, it was observed that AMH was often increased in comparison with $\Delta 4$ (100% vs. 31.8%). Women, who had an increased $\Delta 4$, did not frequently show an increased AMH when compared with those who had a normal $\Delta 4$. Therefore, $\Delta 4$ does not appear to be an indicator associated with AMH. However, the above finding is in contrast with the known literature which supports that $\Delta 4$ is related to AMH (7).

AMH was more increased compared with DHEAS (76.3% vs. 23.7%). Most of the women, who had increased AMH, had normal DHEAS levels. Also, when AMH was within normal ranges, almost always DHEAS was normal. On the other hand, when DHEAS was increased, AMH was slightly more increased. This result confirms the known literature (40).

Finally, after the evaluation of the basic parameters for PCOS, we found that all parameters were rarely simultaneously increased. Some parameters were increased; more frequently AMH. Specifically, AMH was increased in 76.3% of the sample, T in 50%, $\Delta 4$ in 31.8%, DHEAS in 23.7%, LH in 21%, FSH in 18.4%, ratio: LH/FSH in 18.4% and the glucose level was increased in 15.8% of our sample. Therefore, according to these findings, the assay of AMH is more important in order to investigate PCOS, seconded by the measurement of T. This conclusion is consistent with recent studies suggesting AMH as a key indicator of PCOS (41). However, an international consensus is needed to integrate AMH in the diagnostic criteria of PCOS (42).

In conclusion, according to the results of our study, AMH is a reliable indicator of PCOS. Often, two markers may be present and simultaneously increased, such as AMH and T or AMH and LH. Smoking, alcohol consumption, obesity and diabetes were not associated with AMH. In contrast, T and LH were shown to correlate with AMH. Indeed, unlike LH, FSH is not shown to be increased in PCOS.

Some studies, questioning the reliability and usefulness of the measurement of AMH, placed a greater emphasis on the LH/FSH ratio and on T. However, when comparing all biological and hormonal indicators it was shown that AMH was increased in two of three women in a sample, T in half the females and the other androgens ($\Delta 4$ and DHEAS) were increased in one in three women. Moreover, FSH, LH, the LH/FSH ratio and the glucose levels were more often normal. Therefore, in this study, AMH is the most important indicator of PCOS with a sensitivity of 76.3%. Nevertheless, this result should be validated by further studies with a higher number of participants and an international consensus is necessary to integrate AMH in the diagnostic criteria of PCOS.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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