ORIGINAL INVESTIGATION

Sensitivity and specificity of anti-mülerian hormone in the diagnosis of polycystic ovary syndrome in a macedonian population of women of reproductive age: a cross-sectional study

Sasha Jovanovska Mishevska[1], Brankica Krstevska[1], Gordana Pemovska[1], Tatjana Milenkovic[1], Iskra Bitoska[1], Aleksandra Atanasova Boshku[2]

1University Clinic of Endocrinology, Diabetes and Metabolic Disorders, Skopje, Macedonia
2University Clinic of Gynecology and Obstetrics, Skopje, Macedonia

Corresponding author:
Sasha Jovanovska Mishevska, University Clinic of Endocrinology, Diabetes and Metabolic Disorders Skopje, Vodnjanska 17, 1000 Skopje, Macedonia
e-mail: misevskas@yahoo.com

DOI: 10.21040/eom/2016.2.2.2.

Received: May 20th 2016
Accepted: June 5th 2016
Published: June 15th 2016

Funding: None.
Conflict of interest statement: The authors declare that they have no conflict of interest.
Data Availability Statement: All relevant data are within the paper.

Abstract

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine disorders in women of reproductive age, characterized by the association of polycystic ovaries, hyperandrogenism and chronic anovulation. Excessive ovarian production of anti-mülerian hormone (AMH), secreted by the excess of growing follicles, is now considered an important feature of PCOS, with an increasing number of evidence in the last decade on the role of AMH in the pathogenesis of the syndrome. The aim of this study was to determine the sensitivity and specificity of AMH in the diagnosis of PCOS, as well as the association of AMH with other components of the syndrome. A cross-sectional study of clinical, hormonal and biochemical markers in 60 patients with PCOS and 30 controls was conducted. There was a statistically significant difference of AMH values between the groups, with an almost 5-fold increase in circulating AMH levels in women with PCOS compared with those without the syndrome. Positive significant correlation of AMH values with the duration of the menstrual cycle, as well as a significant correlation with testosterone levels and negative significant correlation with the levels of follicle stimulating hormone were observed. Measurement of serum AMH levels as a diagnostic modality of PCOS showed high sensitivity and specificity. Optimal specificity and sensitivity were achieved at the cut-off level of 5 ng/ml offering sensitivity of 82.76% and specificity of 88.89 % with a positive predictive value of 94.12%. This study showed that AMH could be used as an alternative diagnostic tool in PCOS patients.

Key words: anti-mülerian hormone, polycystic ovary syndrome, chronic anovulation, follicle arrest, ultrasonography

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine disorders in women of reproductive age, characterized by the association of polycystic ovaries, hyperandrogenism and chronic anovulation [1,2]. The incidence of PCOS in premenopausal women is 5-10% [3]. Although PCOS is the most frequent endocrine disorder in women of reproductive age, the diagnosis of the syndrome remains one of the most challenging issues in endocrinology, gynecology and reproductive medicine. PCOS is a diagnosis of exclusion and is defined by the Rotterdam classification from 2003 requiring at least 2 out of 3 criteria: oligo and/or anovulation, clinical and/or biochemical hyperandrogenism and polycystic ovaries on ultrasound [4].

Excessive ovarian production of anti-mülerian hormone (AMH), secreted by the excess of growing follicles, is now considered as an important feature of PCOS, with increasing evidence in the last decade on the role of AMH in the pathogenesis of the syndrome [5]. AMH is a dimeric glycoprotein, a member of the transforming growth factor β (TGF-β) family of growth and differentiation factors [6]. In women, AMH expression is restricted to one cell type: the granulosa cells. AMH is expressed as soon as the primordial follicles are recruited to grow into small preantral follicles and its highest expression is observed in preantral and small antral follicles. AMH expression then decreases with the selection of follicles for dominance and is no longer expressed during the FSH-dependent stages of follicular growth [5]. Women with PCOS have an increased number of small follicles in the preantral and antral stage resulting from chronic hyperandrogenemia, and therefore, their AMH serum concentrations are higher than their counterpart [7]. AMH levels have been shown to have a positive correlation with the individual features of PCOS, including LH concentrations, testosterone levels, mean ovarian volume and the number of ovarian follicles [8].

Since the level of AMH reflects the number of growing follicles, serum levels of the hormone can be used as a marker of the degree of ovarian follicular arrest. The aim of this study was to determine the sensitivity and specificity of AMH in the diagnosis of PCOS, as well as the association of AMH with the other components of the syndrome.

2. Material and methods

A cross-sectional study was conducted at the University Clinic of Endocrinology, Diabetes and Metabolic Disorders in Skopje, Macedonia, in the period between May 2015 and May 2016. Sixty consecutive patients with PCOS were enrolled in the study and 30 normo-ovulatory women with regular menstrual cycles, without clinical or biochemical signs of hyperandrogenism and no prior known endocrine diseases were used as controls. The local medical ethics committee approved the study and all participants gave informed consent before the onset of the study. The inclusion criteria were: (a) presence of both ovaries, (b) no use of hormone therapy in the 3 months preceding the study, (c) no history of premature ovarian failure, (d) no previous ovarian surgery, (e) no exposure to cytotoxic drugs or pelvic radiation therapy. PCOS was diagnosed according to the criteria from The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group [4]. Patients presenting with thyroid disorders or neoplastic causes of hyperandrogenemia such as androgen-secreting tumors, congenital adrenal hyperplasia and Cushing's syndrome were excluded from the study.

Hormonal parameters were assessed in the follicular phase of the menstrual cycle or at any given day in women with absent menstrual cycles in the previous two or more months. Blood samples for hormonal and biochemical analyses were obtained by venepuncture between 08:00 and 10:00 h after a 12-hour overnight fast. Anthropometrical measurements and clinical assessment of signs of hyperandrogenism were conducted at the visit. Transvaginal ultrasound scan of the ovaries was performed at the University clinic of Gynaecology using a 6 MHz transducer in order to determine the total number of early antral follicles.

Serum estradiol (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T), androstenedione (A), dehidroepiandrostenedione-sulphate (DHEA-S) and prolactin (PRL) levels were measured

by electrochemiluminiscence immunoassay on a Roche Elecsys 1010/2010 automated immunoassay analyzer. Sex hormone binding globulin (SHBG) measurements were performed by enzyme-linked immunosorbent assay on an IMX Abbott semiautomatic analyzer. AMH serum values were determined by AMH Gen II ELISA technic, using enzymatic amplified two-sided assay, an ultra-sensitive enzyme-linked immunoassorbent assay. Free androgen index (FAI) was calculated using the standard formula testosterone/SHBG × 100.

All statistical procedures were performed using the StatSoft Statistica, version 7 software. Statistical significance was set at $p < 0.05$. Normality of distribution was evaluated with the one-sample Kolmogorov-Smirnoff test. Comparisons of means were performed with one-way ANOVA and general linear model multi-variance. Correlations were evaluated with calculation of the Spearman coefficient.

Table 1. Clinical and ultrasonographic characteristic of women with PCOS and controls

	PCOS (n=60)	Controls (n=30)	p value
PCO-US	0,77 ± 0,42	0,1 ± 0,31	<0,01
Age (years)	23,65 ± 4,36	25 ± 4,92	0,27
BMI (kg/m2)	26,44 ± 6,48	23,78 ± 5,04	0,11
Hirsutism	0,72 ± 0,345	0,1 ± 0,31	<0,01
Acne	0,23 ± 0,42	0,17 ± 0,39	0,62
Waist circumference(cm)	0,81 ± 17,72	84,84 ± 15,93	0,25
Hip circumference (cm)	105,1 ± 13,63	117,72 ± 17,7	0,63
Oligomenorrhea (days)	104,15 ± 102,67	32 ± 11,09	<0,01
BP systolic (mmHg)	113,46 ± 17,95	115,52 ± 7,95	0,63
BP diastolic (mmHg)	72,30 ± 10,92	78,5 ± 4,56	0,35

PCO-US – presence of polycystic ovaries detected by ultrasound; BMI – Body mass index; BP – blood pressure

Table 2. Hormonal characteristic of women with PCOS and controls

	PCOS (n=60)	Control (n=30)	p value
FSH (mU/ml)	5,83 ± 2,01	6,18 ± 1,65	0,49
LH (mU/ml)	11,93 ± 8,33	4,04 ± 1,61	<0,01
LH/FSH	2,16 ± 1,27	0,66 ± 0,24	<0,01
estradiol (pg/ml)	73,74 ± 61,88	45,31 ± 11,16	0,06
PRL (ng/ml)	13,08 ± 5,93	12,84 ± 5,9	0,88
testosterone (nmol/l)	2,21 ± 0,71	1,09 ± 0,46	<0,01
Free androgen index	1,13 ± 0,53	0,43 ± 0,24	<0,01
DHEA-S (μmol/l)	9,08 ± 2,92	5,53 ± 3,31	<0,01
androstenedione (ng/ml)	3,67 ± 1,71	2,77 ± 1,27	0,08
SHBG (nmol/L)	40,38 ± 23.36	56,00 ± 24.43	<0.016
AMH (ng/ml)	13,23 ± 7,04	2,9 ± 1,61	<0,01

FSH – follicle stimulating hormone; LH – luteinizing hormone; PRL – prolactin, DHEA-S – dehidroepiandrostenedione-sulphate; SHBG - Sex hormone binding globulin; AMH – anti-müllerian hormone

3. Results

Of the 90 subjects who participated in this study, 60 were diagnosed with PCOS according to the Rotterdam diagnostic criteria and the control group consisted of 30 subjects. The basic clinical data for both patient groups (group with PCOS and control group) are shown in Table 1. As depicted by the definition of PCOS, there were statistically significant differences between the groups in the presence of polycystic ovaries detected by ultrasound (PCO-US) (79% with polycystic ovaries in the PCOS group vs 7% in the control group, $p<0.01$), the presence and level of hirsutism (81% with moderate to severe hirsutism in the PCOS group vs. 10.6% in the control group, $p<0.01$) and the duration of menstrual cycle (104.15 ± 102.67 vs. 32 ± 11.09 days between menstrual cycles, $p<0.01$). The median age in the PCOS group was lower than the age of subjects in the control group, but this difference did not reach statistical significance. The mean body mass index (BMI), waist and hip circumferences and the systolic and diastolic blood pressure measurements were comparable in both groups.

AMH levels were measurable in all participants, with values ranging from 0.67 to 25 ng/ml. There was a statistically significant difference of AMH values between the groups, with an almost 5-fold increase in circulating AMH levels in women with PCOS compared with those without the syndrome. There were also statistically significant differences between the PCOS and the control group in median/mean LH, and LH/FSH ratio levels, as well as in the classical biochemical markers of hyperandrogenism (total testosterone, free testosterone, bioavailable testosterone, DHEA-S and SHBG levels). The mean FSH, estradiol, prolactin and androstenedione levels were comparable in both groups. The hormonal profiles for both patient groups are shown in Table 2.

Table 3. Correlations of AMH with different parameters in the PCOS group reaching statistically significant values

Correlations (AMH) Marked correlations are significant at p < 0,05						
	Mean	Std.Dv	r(X,Y)	r^2	t	p
Oligomenorrhea (days)	104,15	102,68				
AMH (ng/ml)	13,57	6,84	0,30	0,09	2,20	0,03
FSH (mU/ml)	5,83	2,01				
AMH (ng/ml)	13,23	7,04	-0,30	0,09	-2,38	0,02
testosterone (nmol/l)	2,21	0,71				
AMH (ng/ml)	13,23	7,04	-0,27	0,07	-2,11	0,04
testosterone (ng/dl)	63,60	20,40				
AMH (ng/ml)	13,23	7,04	-0,27	0,07	-2,11	0,04
free testosterone (ng/dl)	1,13	0,53				
AMH (ng/ml)	13,17	7,11	-0,28	0,08	-2,16	0,04
bioavailable testosterone (ng/dl)	26,51	12,36				
AMH (ng/ml)	13,17	7,11	-0,28	0,08	-2,16	0,03

AMH – anti-müllerian hormone, FSH – follicle stimulating hormone

Table 4. Sensitivity and specificity of AMH as a marker in the diagnosis of PCOS

AMH	Value	95% CI
Sensitivity	82.76%	70.57% to 91.41%
Specificity	88.89 %	70.84% to 97.65%
Positive Predictive Value	94.12%	83.76% to 98.77%
Negative Predictive Value	70.59 %	52.52% to 84.90%

CI – confidence interval

Spearman correlation results in the group with PCOS showed a positive significant correlation of AMH values with the duration of the menstrual cycle in days, as well as a significant correlation with the testosterone levels (both total testosterone, free testosterone and bioavailable testosterone indexes). Correspondingly, a negative significant correlation of AMH with FSH ($r = -0.3$, $P < 0.03$) was found. Correlations of AMH with different parameters in the PCOS group reaching statistically significant values are shown in Table 3.

An AMH cut-off level of 5 ng/ml was obtained using a Receiver Operating Characteristic (ROC) procedure. Based upon this cut-off value, the sensitivity and specificity of AMH as a marker in the diagnosis of PCOS was calculated, as well as its positive and negative predictive value and the 95% confidence intervals, given in Table 4.

4. Discussion

In our study, PCOS patients did not have higher BMI than those without the syndrome, which is different from that of typical PCOS populations [9]. We found no correlation between AMH and BMI, in accordance with the earlier observations of Pigny and colleagues, who also found that BMI did not influence circulating AMH concentrations in women with PCOS [10]. Nardo and colleagues also found no relationship between AMH and BMI, which was explained by the differences in study populations and clinical settings, mainly the pre-selection of patients based on BMI cut-offs, which was the case in their study [11].

Regarding clinical features, we demonstrated a statistically significant positive correlation between serum AMH and cycle length in days. Cycle length can be considered as a reflection of the degree of anovulation, therefore reflecting the severity of the disorder. We can say that not only is AMH elevated in women with PCOS, but it also correlates with the severity of the syndrome. A recent study reported that the strongest group difference for AMH levels was found in the group with severe PCOS patients versus controls [12].

It has been shown that the serum concentrations of AMH are proportional to the number of small antral follicles in the ovary [13]. Ovarian dysfunction in women with PCOS is characterized by the arrest of follicular maturation and disturbed process of selection of a dominant follicle. Women with PCOS have a two to six times greater number of follicles (primary, secondary and antral) in the ovaries, as a result of the chronic hyperandrogenemic state [14]. The defective selection mechanism results in an accumulation of small antral follicles, which contribute significantly to the production of AMH. Accordingly, a 2 to 3-fold increase in the serum concentrations of AMH found in many studies, has been explained as a direct reflection of the increased number of early antral follicles [15] . This observation has been confirmed by our results.

In 2007, Pellat et al. reported that in women with PCOS, the production of AMH from the granulosa cells is increased by 75% when compared to controls. According to the authors, these high AMH levels are due not only to the increased number of growing follicles, but are also due to an intrinsic aberrant follicular function. The excess of AMH most likely acts through paracrine pathways in the process of follicular arrest. It has been suggested that AMH inhibits the recruitment of the primary follicle and diminishes the response of the selected follicle to FSH stimulation, thus interfering with the process of maturation into a dominant follicle [6]. The functional role of AMH in early follicular growth has been characterized by the study of "knocked out" models for the AMH gene [16]. When there is no AMH, primordial follicles are recruited faster, resulting in more growing follicles. Therefore, AMH has an inhibitory effect on early follicular recruitment, preventing the entry of primordial follicles into the growing pool, and thus, premature exhaustion of follicles [5]. AMH also has an inhibitory effect on cyclic follicular recruitment in vivo by reducing the follicle sensitivity to FSH. AMH also reduces the number of LH receptors in granulosa cells, also an FSH induced process. Thus, it is clear that AMH is involved in the regulation of follicular growth initiation and in the threshold for follicle FSH sensitivity [5]. The negative correlation of AMH with FSH values in our cohort supports these findings.

Measurement of serum AMH levels as a diagnostic modality of PCOS turned out to have a high sensitivity and specificity. Optimal specificity and sensitivity were achieved at a cut-off level of 5 ng/ml offering a

sensitivity of 82.76% and specificity of 88.89%, with a positive predictive value of 94.12%. This study showed that AMH could be used as an alternative diagnostic tool in PCOS patients. Pigny et al. found that the specificity and sensitivity of serum AMH measurement reached 92% and 67 %, respectively [17]. Lin et al. obtained a cut-off AMH level of 7.3 ng/mL, giving 76% specificity and 70 % sensitivity to predict PCOS [18]. Serum AMH assay has many benefits over other markers of ovarian reserve. First, its plasmatic level is quite stable from one cycle to another and throughout the same cycle since the dominant follicle and corpus luteum do not secrete AMH [5]. Besides its minor variability, serum AMH is also useful when the antral follicle count (AFC) cannot be done such as in obese, virgin or poorly echogenic patients [19]. Moreover, serum AMH level is rather independent from the hypothalamic pituitary axis; therefore, it is not modified in pathologies such as hyperprolactinemia, functional hypothalamic amenorrhea or in incomplete and recent hypogonadotropic hypogonadism, providing serum FSH levels remain normal or sub-normal [20].

5. Conclusion

As a diagnostic marker, AMH measurement has been found to offer a relatively high specificity and sensitivity for PCOS. Given its strong implication in the pathophysiology of PCOS, AMH measurement would theoretically be more accurate than ultrasound, as it also reflects the excess of small antral follicles non-visible on ultrasound. In situations where accurate ultrasound data are not available, AMH could be used as an alternative to follicle count as a diagnostic criterion for PCOS.

Author contributions

SJM gave the idea for the article, wrote the paper, participated in drafting the article and gave her final approval. BK and GP participated in data acquisition, drafting of the article and gave their final approval. IB performed statistical analyses and gave their final approval. AAB and TM critically revised the manuscript, gave suggestions regarding data analysis and presentation and gave their final approval.

References

1. Franks S. Polycystic Ovary Syndrome. N Engl J Med 1995;333:853–61.
http://dx.doi.org/10.1056/NEJM199509283331307

2. Futterweit W. Polycystic ovary syndrome: clinical perspectives and management. Obstet Gynecol Surv 1999;54:403–13.
http://dx.doi.org/10.1097/00006254-199906000-00024

3. Dunaif A, Thomas A. Current concepts in the polycystic ovary syndrome. Annu Rev Med 2001;52:401–19.
http://dx.doi.org/10.1146/annurev.med.52.1.401

4. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19–25.
http://dx.doi.org/10.1016/j.fertnstert.2003.10.004

5. Dumont A, Robin G, Catteau-Jonard S, Dewailly D. Role of Anti-Müllerian Hormone in pathophysiology, diagnosis and treatment of Polycystic Ovary Syndrome: a review. Reprod Biol Endocrinol 2015;13:137.
http://dx.doi.org/10.1186/s12958-015-0134-9

6. Visser JA, de Jong FH, Laven JSE, Themmen APN. Anti-Müllerian hormone: a new marker for ovarian function. Reproduction 2006;131:1–9.
http://dx.doi.org/10.1530/rep.1.00529

7. Homburg R, Crawford G. The role of AMH in anovulation associated with PCOS: a hypothesis. Hum Reprod 2014;29:1117–21.
http://dx.doi.org/10.1093/humrep/deu076

8. Laven JSE, Mulders AGMGJ, Visser JA, Themmen AP, De Jong FH, Fauser BCJM. Anti-Mullerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. J Clin Endocrinol Metab 2004;89:318–23.
http://dx.doi.org/10.1210/jc.2003-030932

9. Chang WY, Knochenhauer ES, Bartolucci AA, Azziz R. Phenotypic spectrum of polycystic ovary syndrome: clinical and biochemical characterization of the three major clinical subgroups. Fertil Steril 2016;83:1717–23.
http://dx.doi.org/10.1016/j.fertnstert.2005.01.096

10. Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, S. Jonard, et al. Elevated serum level of anti-mullerian 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.
http://dx.doi.org/10.1210/jc.2003-030727

11. Nardo LG, Yates AP, Roberts SA, Pemberton P, Laing I. The relationships between AMH, androgens, insulin resistance and basal ovarian follicular status in non-obese subfertile women with and without polycystic ovary syndrome. Hum Reprod 2009;24:2917–23. http://dx.doi.org/10.1093/humrep/dep225

12. Koninger A, Koch L, Edimiris P, Enekwe A, Nagarajah J, Kasimir-Bauer S, et al. Anti-Mullerian Hormone: an indicator for the severity of polycystic ovarian syndrome. Arch Gynecol Obstet 2014;290:1023–30. http://dx.doi.org/10.1007/s00404-014-3317-2

13. Kevenaar ME, Meerasahib MF, Kramer P, Van De Lang-Born BMN, De Jong FH, Groome NP, et al.Serum anti-Mullerian hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology 2006;147:3228–34. http://dx.doi.org/10.1210/en.2005-1588

14. Norman RJ, Dewailly D, Legro RA, Hickey TE. Polycystic ovary syndrome. Lancet (London, England) 2007;370:685–97. http://dx.doi.org/10.1016/S0140-6736(07)61345-2

15. Broer SL, Broekmans FJM, Laven JSE, Fauser BCJM. Anti-Mullerian hormone: ovarian reserve testing and its potential clinical implications. Hum Reprod Update 2014;20:688–701. http://dx.doi.org/10.1093/humupd/dmu020

16. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Kumar TR, Matzuk MM, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology 2001;142:4891–99.

17. Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:941–945. http://dx.doi.org/10.1210/jc.2005-2076

18. Lin YH, Chiu WC, Wu CH, Tzeng CR, Hsu CS, Hsu MI. Antimullerian hormone and polycystic ovary syndrome. Fertil Steril 2011;96:230–35. http://dx.doi.org/10.1016/j.fertnstert.2011.04.003

19. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, et al. The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update 2014;20:370–85. http://dx.doi.org/10.1093/humupd/dmt062

20. Tran ND, Cedars MI, Rosen MP. The role of anti-mullerian hormone (AMH) in assessing ovarian reserve. J Clin Endocrinol Metab 2011;96:3609–14. http://dx.doi.org/10.1210/jc.2011-0368