VIA MEDICA

REVIEW PAPER / GYNECOLOGY

Ginekologia Polska
2022, vol. 93, no. 9, 756–760

ISSN 0017–0011, e-ISSN 2543–6767

DOI 10.5603/GP.a2022.0075

Anti-Mullerian hormone levels in girls and adolescents

Iwona J. Czech, Agnieszka Drosdzol-Cop

Chair and Department of Gynecology, Obstetrics and Oncological Gynecology, Medical University of Silesia in Katowice, Poland

ABSTRACT

Anti-Mullerian hormone (AMH) is a homodimeric glycoprotein which belongs to the TGF-beta superfamily of growth and differentiation factors. There are few studies that present AMH concentrations in premenarcheal and early postmenarcheal girls. The aim of this study is to evaluate AMH levels in girls. A serum AMH increase of 1 pmol/L is related with a higher possibility of menarche occurrence. AMH levels are significantly higher in postmenarcheal girls than in prepubertal girls.

Key words: Anti-Mullerian hormone; puberty; adolescents; pediatric endocrinology

Ginekologia Polska 2022; 93, 9: 756–760

INTRODUCTION

Anti-Mullerian hormone (AMH) is a homodimeric glycoprotein, with a molecular weight of 140 kDa, which belongs to the TGF-beta superfamily of growth and differentiation factors. The gene encoding AMH is located on the short arm of chromosome 19 in humans, band 19p 13.3.

It plays major role in developing male reproductive tract by activating the regression of male fetal Mullerian ducts [1]. In females it is produced by the granulosa cells of primary, preantral, and early antral follicles. The highest concentration of AMH is shown by small antral follicles (2–4 mm in diameter). AMH has two functions during follicular development. Firstly, it inhibits the transition of primordial follicles to the mature phase and it is through this mechanism that it plays a role in the regulation of the number of follicles resting in the reservoir of primordial follicles. Secondly, it decreases the FSH-sensitivity of the follicles, playing a role in the process of follicle selection.

In recent years, AMH has gained importance in endocrinologic gynecology. Serum concentration of AMH are much lower than the concentration in the follicle, although it correlates with it well. The AMH level determination can be performed independently of the phase of the menstrual cycle. A decrease in the number of follicles correlates with a decrease in AMH concentration. Therefore, serum AMH determination is mainly used to assess the ovarian reserve reflecting AFC and therefore it may be used as a marker during ovarian stimulation strategies [2]. The concentrations of AMH reflect the aging process of the ovary. Reduced AMH levels are also observed in the premature ovarian failure. Moreover, AMH may be a useful marker of granulosa-cell tumors (folliculoma) and their recurrence. In these clinical situations, AMH levels can be very high, and they correlate with tumor size. What is more, in women with polycystic ovarian syndrome (PCOS) AMH levels are observed to be higher than in healthy females. Although determination of AMH level is not necessary for the diagnosis of PCOS, it may be a useful and valuable marker.

There are few studies that present AMH concentrations in premenarcheal and early postmenarcheal girls. According to such studies, AMH levels rise during infancy and are stable from childhood to early adolescence, as they slightly decline from 9 to 15 years of age and then increase with the peak levels around 25 years of age [3–6]. Hagen et al. have presented data that AMH levels increase three years prior to the start of puberty, while decreasing after pubertal onset by 30% during the first two years [4, 7]. Lee et al. [8] have shown that AMH levels increase at 6-8 years of age and reach a peak during late adolescence.

The aim of this study is to evaluate AMH levels in girls.

MATERIAL AND METHODS

The subjects of this study were consecutive girls who were referred to the Pediatric Endocrinology Depart-

Corresponding author:
Iwona J. Czech
Chair and Department of Gynecology, Obstetrics and Oncological Gynecology, Medical University of Silesia in Katowice, 87 Markiefki St, 40–211 Katowice, Poland
e-mail: iwonaczech232@gmail.com

Received: 29.03.2022 Accepted: 30.03.2022 Early publication date: 16.08.2022

ment and Outpatient Clinic at Upper Silesian Child Health Center between 1 June 2019 and 31 March 2020 (n = 100). The exclusion criteria were:

— Thyroid, prolactin, androgen, cortisol level disorders
— Chronic systemic illness
— Eating disorders
— Smoking or drug or alcohol use
— Taking medications known to interfere with reproductive hormones/receiving hormonal contraception.

Eighteen girls were excluded from the study and 82 girls were enrolled to undergo further research. Demographic and clinical data were retrieved from medical records.

The study was approved by the Ethics Committee of Medical University of Silesia (KNW/0022/KB1/3/19).

Clinical data

Anthropometric measurements included weight and height measurements. Body mass index (BMI) was calculated for each girl using the standard formula of weight (kg) divided by height (m) squared. The BMI Z-score and BMI percentile for each girl were calculated using The Pediatric Z-score Calculator, which is available on the website of The Children's Hospital of Philadelphia, Research Institute (https://zscore.research.chop.edu/calcbmi.php) and is based on the Center for Disease Control (CDC) growth charts.

Biochemical measurements

Blood samples were drawn from an antecubital vein between 8 am and 10 am after an 8-hour fast for luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone (TTE), inhibin B and anti-Mullerian hormone (AMH). The samples were centrifuged and stored at -20°C until analyses were conducted. TTE, LH, FSH, and AMH were measured with the electrochemiluminescence "ECLIA" method (Elecys, Roche Diagnostics GmbH, Mannheim). Inhibin B was measured with an enzyme-linked immunosorbent assay (ELISA Genie ELISA).

In postmenarcheal girls, the samples were drawn between the 2nd and 5th day of their menstrual cycle.

All measurements were performed in the Laboratory of the Upper Silesian Child Health Center, Katowice, Poland.

Pelvic ultrasound

The subjects underwent a transabdominal pelvic ultrasound. The length, width and volume of the ovaries were measured. Uterus and cervix measurements were also taken.

Statistical analysis

Statistical analysis was performed using StatSoft Statistica version 13.3 software. Quantitative variables are presented as a mean and standard deviation (SD) or median and interquartile range (IQR). The qualitative variables are presented as an absolute value and/or percentage. The between-group differences for quantitative variables were verified using a parametric (t-test or ANOVA) or non-parametric tests (U Mann-Whitney or Kruskal-Wallis), with previous verification of their distribution by the Shapiro-Wilk or Smirnov-Kolmogorov tests. In the case of qualitative variables, the chi-square test or Fisher's exact test was used. A receiver-operating characteristic (ROC) curve analysis was used to assess the diagnostic accuracy of AMH predicting menarche. Logistic regression analysis was conducted in order to assess the power of AMH increase in menarche occurrence. A p value of < 0.05 was considered significant.

RESULTS

A total of 82 healthy girls and adolescents were included. The median age was 142 months (11 years and 10 months) (IQR 111-180). The youngest subject was 87 months old (7 years and 3 months) and the oldest was 215 months old (17 years and 11 months). In addition, 30 girls were after menarche, while 52 girls had not menstruated yet. The clinical, demographic and ultrasound characteristics of the patients are presented in Table 1.

The mean AMH level was 30.47 ± 21 pmol/L. There were significant differences between the group of premenarcheal and postmenarcheal girls (25.2 pmol/L vs 39.6 pmol/L, p = 0.01). Mean TTE, LH, FSH, inhibin B and AMH levels are presented in Table 2.

The variations of AMH depending on age are presented in Figure 1.

ROC analysis was used to determine the cutoff level of AMH. The value of AMH that indicates menarche was

Table 1. Clinical and ultrasound characteristics of the study group

	TOTAL (n = 82)	Premenarcheal (n = 52)	Postmenarcheal (n = 30)
Age [months]	142 (IQR 111–180)	111.5 (IQR 106–140)	194 (IQR 177–205)
BMI	20.24 ± 4.58	17.93 ± 2.8	24.23 ± 4.35
BMI Z-score	0.41 ± 1	0.22 ± 1	0.75 ± 0.95
BMI percentile	60.83 ± 30.22	55.4 ± 30.22	70.23 ± 28.29
Mean ovarian volume [mL]	2.72 ± 3.09	0.82 ± 0.7	6.02 ± 2.85

BMI — body mass index; IQR — interquartile range

Table 2. Laboratory data and comparison of the groups

	TOTAL (n = 82)	Premenarcheal (n = 52)	Postmenarcheal (n = 30)	p-value
AMH [pmol/L]	30.47 ± 21	25.2 ± 16.93	39.59 ± 19.67	p = 0.01
LH [mIU/mL]	4.76 ± 6.6	1.51 ± 4.16	10.38 ± 6.32	p < 0.001
FSH [mIU/mL]	3.91 ± 4	3.31 ± 4.66	4.92 ± 2.23	p < 0.001
TTE [ng/dL]	18.62 ± 21	6.67 ± 9.06	39.33 ± 19.67	p < 0.001
INHIBIN B [jednostka??]	0.45 ± 2	0.31 ± 1.38	0.69 ± 2.8	p = 0.8

AMH — Anti-Mullerian hormone; LH — luteinizing hormone; FSH — follicle-stimulating hormone; TTE — total testosterone

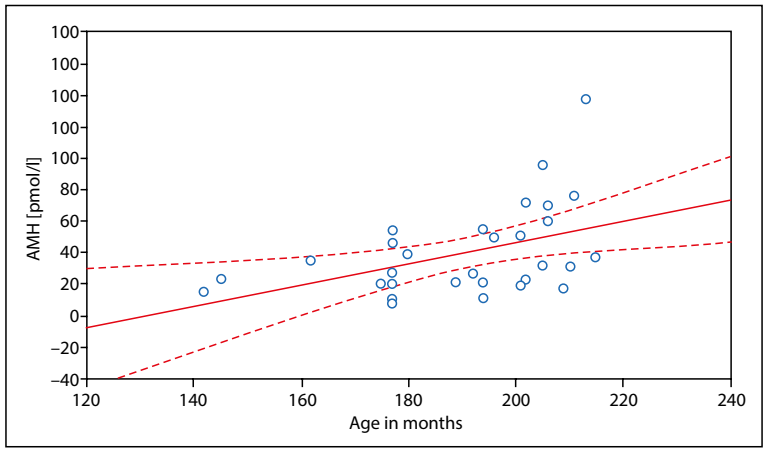

Figure 1. Variations of Anti-Mullerian hormone (AMH) depending on age. AMH was significantly associated with age (p < 0.05)

determined as 30.63 pmol/L with 53% sensitivity and 73% specificity (p = 0.007). The area under the ROC curve was determined as 0.666 (CI 0.545–0.787) (Fig. 2).

Regression analysis was performed in order to assess the relationship between an increase in AMH levels and menarche occurrence. An increase of 1 pmol/L of AMH resulted in 1.03 times higher possibility of menarche (p < 0.005).

The mean AMH level in the group of girls who had been menstruating for more than 24 months (n = 19) was 45.53 ± 29.75 pmol/L. Mean AMH level in the group of girls who had been menstruating for 24 months or less (n = 11) was 29.33 ± 24.1, p > 0.05. In addition, there were no statistically important differences in AMH levels between premenarcheal girls and girls who had been menstruating for less than 24 months.

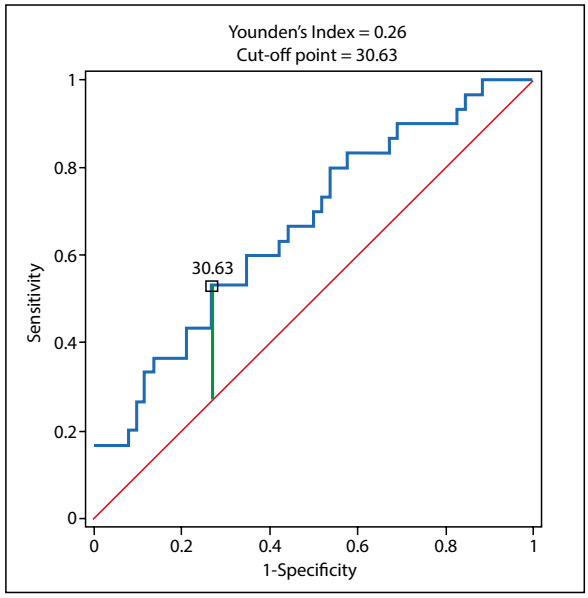

Figure 2. Receiver-operating characteristic curve of Anti-Mullerian hormone (pmol/L)

DISCUSSION

In this single-center study we sought to investigate the fluctuations of AMH in the group of healthy pre- and postmenarcheal girls. There are few studies that report the variations of AMH during childhood and puberty. Ortega et al. [9] report that healthy, early postmenarcheal girls have an average AMH level of 37.13 ± 2.14 pmol/L (5.2 ± 0.3 ng/mL).

Studies describing the variations of AMH show that its levels slightly rise until about 9 years of age, then there is a slight

decline from 9 to about 15 years of age and then they increase [5, 6, 10]. In our study age was significantly associated with AMH levels, there was a significant raise in levels observed from the age of 168 months of age (14 years).

As AMH is considered one of the indicators of precocious puberty and may be useful in differentiation of central precocious puberty (CPP) and premature thelarche (PT), it is important to evaluate the fluctuations of this hormone in healthy girls [11, 12]. Sahin et al. [11] found out that the AMH levels in patients with CPP were lower than in patients with PT. They determined the level of AMH that defines CPP as 9.03 pmol/L. Chen et al. [8] remain consistent with this finding. In this study we focused on investigating the range of AMH levels in healthy young females that can serve as a reference for clinicians in differentiating CPP, PT and other pubertal problems with physiological changes. Chen et al. [12] present that AMH levels can be potential biomarkers for distinguishing progression rates in girls with CPP and they can help in the distinction of progressive CPP and less severe CPP. In addition, Efthymiadou et al. [13] found out that AMH concentration is increased in girls with premature adrenarche compared with healthy girls. In contrary, Utriainen et al. [14] found out that girls with premature adrenarche had lower serum AMH concentrations than the control group. Sahin et al. [11] underlined that AMH may be a marker for diagnosis of CPP and PT. These findings show how important is to establish AMH levels in healthy girls to identify potential endocrinologic disorders.

Our findings show that the mean levels of AMH in premenarcheal girls were 25.2 ± 16.93 pmol/L. Savas-Erdeve et al. [15] reported that average AMH levels in a group of 22 prepubertal girls (22 female cases who were prepubertal before the age of 8) were 14.9 ± 6.1 pmol/l (2.1 ± 0.85 ng/mL).

In our study, there were no significant differences found in the girls who had been menstruating for at least two years and girls who had been menstruating for less than two years (45.53 ± 29,75 pmol/L vs 29.33 ± 24.1, $p > 0.05$, $p > 0.05$, respectively). However, these findings may be due to the small number of girls in both subgroups.

The main limitation of this work is the small number of participants, although it is challenging to gather large representative group of healthy minors who present to the outpatient clinic or hospital ward for diagnostics, as many of them meet the exclusion criteria. Moreover, as the formal consent of their parents is needed, even when a minor meets the inclusion criteria, she may not be included to the study.

However, we hope that this study may be useful for clinicians in pediatric and endocrinologic pediatric wards when interpreting the laboratory examinations of their patients, as well as for the authors of further articles on this topic. Understanding the variations in AMH levels in healthy female adolescents may enable early identification of diminished ovarian reserve, risk of developing polycystic ovarian syndrome or premature puberty.

CONCLUSIONS

In conclusion, a serum AMH increase of 1 pmol/L is related with a higher possibility of menarche occurrence. AMH is significantly connected with age. AMH levels are significantly higher in postmenarcheal girls than in prepubertal girls. No differences were found between the AMH levels in girls who had been menstruating for more than 24 months and girls who had been menstruating for less than 24 months.

Acknowledgments

The authors want to thank the nurses of the Pediatric Endocrinology Ward for their support and help in conducting this study, as well as the laboratory diagnosticians from the Laboratory in the Upper Silesian Child Health Center, Katowice, Poland for performing the biochemical assays.

Conflict of interest

All authors declare no conflict of interest.

REFERENCES

1. Josso N, Rey RA, Picard JY. Anti-müllerian hormone: a valuable addition to the toolbox of the pediatric endocrinologist. Int J Endocrinol. 2013; 2013: 674105, doi: 10.1155/2013/674105, indexed in Pubmed: 24382961.
2. Özcan P, Fıçicioğlu C, Ateş S, et al. The cutoff values of serum AMH levels and starting recFSH doses for the individualization of IVF treatment strategies. Gynecol Endocrinol. 2017; 33(6): 467–471, doi: 10.1080/09513590.2017.1294154, indexed in Pubmed: 28277814.
3. Hagen CP, Aksglaede L, Sørensen K, et al. Serum levels of anti-Müllerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. J Clin Endocrinol Metab. 2010; 95(11): 5003–5010, doi: 10.1210/jc.2010-0930, indexed in Pubmed: 20719830.
4. Codner E, Iñiguez G, Hernández IM, et al. Elevated anti-Müllerian hormone (AMH) and inhibin B levels in prepubertal girls with type 1 diabetes mellitus. Clin Endocrinol (Oxf). 2011; 74(1): 73–78, doi: 10.1111/j.1365-2265.2010.03887.x, indexed in Pubmed: 21039723.
5. Kelsey TW, Wright P, Nelson SM, et al. A validated model of serum anti-müllerian hormone from conception to menopause. PLoS One. 2011; 6(7): e22024, doi: 10.1371/journal.pone.0022024, indexed in Pubmed: 21789206.
6. Kelsey TW, Anderson RA, Wright P, et al. Data-driven assessment of the human ovarian reserve. Mol Hum Reprod. 2012; 18(2): 79–87, doi: 10.1093/molehr/gar059, indexed in Pubmed: 21933846.
7. Hagen CP, Aksglaede L, Sørensen K, et al. Individual serum levels of anti-Müllerian hormone in healthy girls persist through childhood and adolescence: a longitudinal cohort study. Hum Reprod. 2012; 27(3): 861–866, doi: 10.1093/humrep/der435, indexed in Pubmed: 22215627.
8. Lee MM, Donahoe PK, Hasegawa T, et al. Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. J Clin Endocrinol Metab. 1996; 81(2): 571–576, doi: 10.1210/jcem.81.2.8636269, indexed in Pubmed: 8636269.
9. Ortega MT, Carlson L, McGrath JA, et al. AMH is Higher Across the Menstrual Cycle in Early Postmenarchal Girls than in Ovulatory Women. J Clin Endocrinol Metab. 2020; 105(4), doi: 10.1210/clinem/dgaa059, indexed in Pubmed: 32016427.
10. Elchuri SV, Patterson BC, Brown MR, et al. Anti-Mullerian hormone levels in American girls by age and race/ethnicity. J Pediatr Endocrinol Metab. 2015; 28(1-2): 189–193, doi: 10.1515/jpem-2014-0242, indexed in Pubmed: 25153582.

11. Sahin NM, Kinik ST, Tekindal MA, et al. AMH levels at central precocious puberty and premature thelarche: is it a parameter? J Pediatr Endocrinol Metab. 2015; 28(11-12): 1351–1356, doi: 10.1515/jpem-2014-0521, indexed in Pubmed: 26226120.
12. Chen T, Wu H, Xie R, et al. Serum Anti-Müllerian Hormone and Inhibin B as Potential Markers for Progressive Central Precocious Puberty in Girls. J Pediatr Adolesc Gynecol. 2017; 30(3): 362–366, doi: 10.1016/j.jpag.2017.01.010, indexed in Pubmed: 28161677.
13. Efthymiadou A, Bogiatzidou M, Kritikou D, et al. Anti-Müllerian Hormone in Girls with Premature Adrenarche: The Impact of Polycystic Ovary Syndrome History in their Mothers. J Pediatr. 2019; 205: 190–194, doi: 10.1016/j.jpeds.2018.09.064, indexed in Pubmed: 30529136.
14. Utriainen P, Jääskeläinen J, Voutilainen R. Serum anti-müllerian hormone concentrations in prepubertal girls with and without premature adrenarche: The influence of body mass index. Horm Res Paediatr. 2010; 74(3): 207–211, doi: 10.1159/000282113, indexed in Pubmed: 20453465.
15. Savas-Erdeve S, Sagsak E, Keskin M, et al. AMH levels in girls with various pubertal problems. J Pediatr Endocrinol Metab. 2017; 30(3): 333–335, doi: 10.1515/jpem-2016-0217, indexed in Pubmed: 28245188.