

Serum Level of Anti-Mullerian Hormone in Adolescent Girls

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Introduction

Anti-Mullerian hormone is produced by granulosa cells surrounding follicles that have undergone recruitment from the primordial follicle pool but have not been selected for dominance (preantral and early antral follicles). For the past 10 years this was a sensitive indicator for ovarian reserve [1,10]. Ovarian reserve (OR) is a term which describes the functional potential of the ovary, which constitutes the size of the ovarian follicle pool and reflects the number and quality of the oocytes which are within it. Assessment of the OR helps in reflecting the reproductive potential of women [4,7,9]. Antral follicle count is another common indicator for ovarian reserve besides AMH. Compared with AFC, AMH is much earlier and more sensitive for assessing follicular pool. AFC is the sum of follicles in both ovaries seen on ultrasound imaging during early follicular phase (Day 2-4)[11]. Antral follicles are defined as those measuring between 2 and 10 mm on a 2-dimensional plane. AFC is easy and quick to carry out, with good inter cycle and inter observer reliability, but has a compromised precision in women with extreme weight and depends on the time of the cycle [2,8,12]. Serum Anti-Mullerian hormone (AMH) and antral follicle count are well established markers of ovarian reserve [3,13].

Although there are many studies dedicated to the issue of ovarian reserve, there are no mechanisms for identifying adolescents with low ovarian reserve and prevent reproductive losses.

The aim of this research is:

1. Individual interpretation of anti-Mullerian hormone levels and chances of conception

2. Determination of the factors that cause diminished ovarian reserve among adolescents in order to create algorithms to prevent disorders in the reproductive and sexual health systems.

Material and Methods

The subjects of the research were 700 adolescent girls of 10-19 years old randomly selected from different regions of Armenia, including Yerevan. Taking into consideration Coronavirus pandemic, announcements about the survey have been regularly posted on social networks ("Facebook", "Instagram"), which provided an opportunity to ensure the representativeness of the sample. Prospective parent participants who had an adolescent between 10 and 19 years of age were e-mailed general information about the present study and a secure link to the parent consent form. The study is a combined cross-sectional and population-based cohort study of Armenian adolescent girls. After collecting specially designed questionnaire data from the 700 adolescent girls, 3 groups were created.

The first group includes 170 (24.3%) adolescent girls of 10-12 years old.

The second group includes 192 (27.4%) adolescent girls of 13-15 years old.

The third group includes 338 (48.3%) adolescent girls of 16-18 years old.

The purpose of conducting the study was to establish the time of pubertal onset and normative data/ individual interpretation of anti-Mullerian hormone levels for adolescents, assess the ovarian reserve and chances of conception, assess the hormonal levels and reproductive and sexual health status and evaluate the factors that cause diminished ovarian reserve among adolescents in Armenia.

Data was collected between May 2020 and October 2020. To analyze the outcomes, we have grouped recorded data into the following sections:

1. Baseline demographic data.
2. Data of menstrual function (menarche, menstrual disorders, history of menstrual irregularity).
3. Data of gynecological disorders (menstrual disturbances, hormonal tests, treatments, operations).
4. Evaluate the correlation between serum AMH and FSH.
5. Thyroid gland and small pelvis diagnostic ultrasonography was performed.

700 adolescent girls from different medical clinics of Armenia are participants of the study.

Prospective parent participants who had an adolescent between 10 and 19 years of age were e-mailed general information about the present study and a secure link to the parent consent form.

Study instruments

The great interest in premature ovarian reserve depletion is explained by the increase in a significant number of infertile women of reproductive age.

1. Depending on the degree of menstrual disturbances, menstrual irregularity, blood samples were drawn from 700 adolescent girls after 8 hours of fasting on day 3 to day 5 of spontaneous menstruation or progestin-induced or combined oral contraceptive-induced menstrual-like reaction for measuring serum concentration of Anti Mullerian Hormone (AMH), Follicle-stimulating hormone (FSH), Luteinizing hormone (LH). All blood samples were drawn between 8: 00 a.m. and 11 a.m.

Serum AMH, FSH levels were determined using the Enzyme-linked Immunosorbent assay (ELISA) method. According to standard practice, all samples were adjusted using a batch-specific correction factor. Adjustments were blinded for ages and stages of pubertal development. All samples were analyzed in the same laboratory, blinded for the technician, for age and pubertal stage.

After the hormonal determination thyroid gland and small pelvis diagnostic ultrasonography has been performed by the Toshiba Aplio 500 (Japan). Measurement of the ovarian volume and the antral follicular counts, were done in all adolescents who were involved in study while doing a transabdominal /transvaginal ultrasonography. Examination was performed in the early follicular phase of the menstrual cycle. All measurements were carried out in the first half of the day (before 13.00).

2. Sexual maturity rating (Tanner staging) in adolescent girls

3. BMI (Body Mass Index) Categories: BMI was calculated using weight (kg)/ height² (m²).

Obesity, particularly the abdominal phenotype, is undoubtedly a useful clinical predictor of metabolic abnormalities (which can be detected in the early stages of PCOS), for that reason glucose and insulin resistance have been determined among girls with metabolic disorders.

Results and Discussion

In comparison with the first group 16 (8.3%) adolescents from the second group and 87 (25.7%) adolescents from the third group were sexually active.

Pregnancy complications occurred in 130 (76.5%) mothers of adolescent girls from the first group, in 150(78.1%) mothers of adolescent girls from the second group and in 282(83.4%) mothers of adolescent girls from the third group.

106 (62.4%) adolescent girls from the first group were born prematurely, 102(53.1%) from the second group and 161(47.6%) adolescent girls from the third group. 36 (21.2%) adolescent girls from the first group were born small for gestational age, 102(53.1%) from the second group and 161(47.6%) adolescent

girls from the third group. In the first group 50.6% adolescents were overweight, 17.6% were obese and 6.5% were underweight.

In the second group 42.2% adolescents were overweight, 20.8% were obese and 12.5 % were underweight.

In the third group 44.1% adolescents were overweight, 23.4% were obese and 6.5% were underweight.

Mental health and metabolic disorders, musculoskeletal problems were mostly observed in the second group. Neurological/ Developmental disorders, spasticity and movement disorders were observed mostly in the first group. Urogenital disorders and urinary tract infection occurred in 90(52.9%) adolescent girls from the first group, in 114(59.4%) adolescents from the second group and in 104(30.8%) adolescents from the third group. (Table 1).

Table 1

Baseline characteristics of studied groups

Characteristics	First group n=170		Second group n=192		Third group n=338	
	n	%	n	%	n	%
Educational level						
High school or lower	170	100	192	100	197	58.3
University	-	-	-	-	52	15.4
Adolescents with disabilities	23	13.5	43	22.4	21	6.2
Have any sexual intercourse						
Yes	-	-	16	8.3	87	25.7
No	170	100	176	91.7	251	74.3
Adolescent girls born from women having complications during pregnancy and delivery						
Preterm birth	106	62.4	102	53.1	161	47.6
Small for Gestational Age	36	21.2	48	25	109	32.2
Childhood Diseases and Disorders						
Mental Health	17	10.0	48	25.0	68	20.1
Musculoskeletal Problems	28	16.5	52	27.1	68	20.1
Neurological Disorders	36	21.2	40	20.8	36	10.7
Urogenital Disorders	90	52.9	114	59.4	104	30.8
Mumps, Rubella	65	38.2	58	30.2	69	20.4
BMI						
Underweight	11	6.5	24	12.5	22	6.5
Normal weight	43	25.3	47	24.5	88	26.0
Overweight	86	50.6	81	42.2	149	44.1
Obesity	30	17.6	40	20.8	79	23.4

In the first group 124 (72.9%) adolescent girls received treatment for vulvovaginitis. Premature sexual maturation was registered in 8(4.7%) cases. 20(11.8%) adolescents were treated for polycystic ovary syndrome for menstrual irregularity. In 15(8.8%) adolescents ovarian cysts were found by ultrasound examination performed for a specific purpose. 3(1.8%) girls were treated by surgical intervention because of ovarian torsion. In 10(5.9%) cases only a follow-up sonography was performed and 5(2.9%) received progesterone to facilitate resolution of cyst and to treat a menstrual disorder. Congenital uterine anomalies were registered in 9(5.3%) cases.

In the second group 154(80.2%) adolescent girls received treatment for vulvovaginitis pelvic inflammatory disease has been registered among 20(10.4%) adolescent girls. Congenital uterine anomalies were registered among 26(13.5%) adolescents. According to the Rotterdam criteria, polycystic ovary syndrome was diagnosed in 61(31.8%) adolescents. In 57(29.7%) adolescents ovarian cysts were found by ultrasound examination performed for a specific purpose. 16(28.1%) girls were treated by surgical intervention because of ovarian torsion, ovarian apoplexy and dermoid cyst. In 13(22.8%) cases only follow-up sonography was done and 28(49.1%) received progesterone to facilitate resolution of cyst and to treat a menstrual disorder. Premature sexual maturation occurred in 14(7.3%) cases.

In the third group 144(42.6%) adolescent girls received treatment for vulvovaginitis. Pelvic inflammatory disease was registered among 50(14.8%) adolescent girls. Polycystic ovary syndrome was diagnosed in 77(22.8%) adolescents. In 59(17.5%) adolescents ovarian cysts were found by ultrasound examination performed for a specific purpose. 19(5.6%) girls were treated by surgical intervention because of ovarian torsion, ovarian apoplexy, endometrioid cyst, paraovarial cyst and dermoid cyst. Bilateral tubectomy was performed in 2(0.6%) cases. In 14(35%) cases only follow-up sonography was performed and 26 (65%) received progesterone to facilitate resolution of cyst and to treat menstrual disorder. Premature sexual maturation occurred in 49(14.5%) and delayed sexual maturation occurred in 26(7.7%) cases. Congenital uterine anomalies were registered among 38(11.2%) adolescents (Table 2).

Serum AMH, FSH, LH was detectable in all samples. Small pelvic ultrasound examination was also done. A total of 469(67%) adolescent girls had a normal AMH level. 652(93.1%) of adolescents demonstrated ongoing pubertal development, whereas the remaining 48(6.9%) did not enter puberty during follow-up. Serum AMH was detected among these adolescents. For Armenian healthy adolescent girls in the age of 10-18, AMH normal range was 2.0-4.0ng/ml. The AFC and the ovarian volume were positively correlated with the level of AMH. We found no correlation between individual AMH level and age at the stage of entering puberty.

Table 2

Prevalence of gynecological diseases and menstrual cycle disorders

Gynecological Issues	First group n=170		Second group n=192		Third group n=338	
	n	%	n	%	n	%
Vulvovaginitis	124	72.9	154	80.2	144	42.6
Pelvic Inflammatory Disease	-	-	20	10.4	50	14.8
Menstrual Cycle Disorders	30	17.4	118	61.5	136	40.2
Sexual maturation						
Premature Sexual Maturation	8	4.7	14	7.3	49	14.5
Delayed Sexual Maturation	-	-	-	-	26	7.7
Polycystic Ovary Syndrome	-	-	61	31.8	77	22.8
Congenital uterine anomalies	9	5.3	26	13.5	38	11.2
Ovarian Cysts						
Functional Cysts	15	8.8	41	21.4	40	11.8
None functional Cysts	6	3.5	16	8.3	19	5.6
Ovarian Cysts Treatment and Management						
Watchful Waiting/ Medication	15	8.8	41	19.8	40	11.8
Surgery for Ovarian Cysts/Lapa- roscopy	3	1.8	16	38.5	19	5.6

In the first group 130(76.5%) adolescents had normal ovarian reserve: AMH level not less than 2.0ng/ml, FSH level not more than 10mIU/ml. AFC was at least 5 in each ovary (10-25). Ovarian volume was not less than 5sm³. Regular menstrual cycle was 28-30 days.

5(2.9%) adolescents had diminished ovarian reserve: AMH level <1.0ng/ml, episodes of increase in FSH>13mIU/ml. AFC was not more than 3 on each ovary. Ovarian volume was from 3 to 5sm³. Among 35(20.6%) adolescents AMH was in low normal range.

In the first group 40(23.5%) adolescents with low normal and with low range were at a high risk of reproductive dysfunction.

In the first group subclinical hypothyroidism revealed in 48(28.2%) adolescents and autoimmune thyroiditis diagnosed in 30(17.6%) adolescents.

There was a negative correlation between serum AMH and TSH levels and no correlation between serum AMH and anti-thyroid peroxidase levels. This result coincides with the results of another researcher [5,6,14].

In the second group 115(59.9%) adolescents had normal ovarian reserve: AMH level not less than 2.0ng/ml, FSH level not more than 10mIU/ml. AFC was at least 5 in each ovary. Ovarian volume was not less than 5sm³. Regular menstrual cycle was 28-30 days.

6(3.1%) adolescents had diminished ovarian reserve: AMH level <1.0ng/ml, episodes of increase in FSH>13mIU/ml. Ovarian volume was from 3 to 5sm³. AFC was not more than 3 on each ovary.

High level of AMH was detected in 61(31.8%) adolescents with PCOS. Serum concentrations of AMH correlated with the AFC and degree of menstrual disturbances, with the severity of both hyperandrogenism and oligoanovulation in women with PCOS.

Low normal range of AMH was detected in 10(5.2%) adolescents. In the second group 77(40.1%) adolescents with high, low normal and with low range were at a high risk of reproductive dysfunction.

In the second group subclinical hypothyroidism was revealed in 72(37.5%) adolescents and autoimmune thyroiditis diagnosed in 68(35.4%) adolescents, which were at a high risk of reproductive losses.

In the third group 224(66.3%) adolescents had normal ovarian reserve. Normal ovarian reserve is characterized: AMH level not less than 1.5ng/ml (1.5-4.0ng/ml), FSH level not more than 10mIU/ml (<10mIU/ml). AFC was at least 5 in each ovary. Ovarian volume was not less than 5cm³. Regular menstrual cycle was 28-30 days.

8(2%) adolescents had diminished ovarian reserve. Diminished ovarian reserve is characterized: AMH level <1.0ng/ml, episodes of increase in FSH>13mIU/ml. AFC was not more than 3 on each ovary (<10). Ovarian volume was from 3 to 5cm³.

High level of AMH was detected in 77(22.8%) adolescents with PCOS. Low normal range of AMH was detected in 26(7.7%) adolescents, low range of AMH was detected in 11(3.3%) adolescents with bilateral oophorectomy. In the third group 114(33.7%) adolescents with high, low normal and with low range were at a high risk of reproductive dysfunction.

In the third group subclinical hypothyroidism was revealed in 123(36.4%) adolescents and autoimmune thyroiditis diagnosed in 116(34.3%) adolescents, which were also at a high risk of reproductive losses (Table 3).

Ovarian reserve in adolescent girls born prematurely was low compared to those of girls born full term. The levels of AMH were lower in the girls born prematurely and Follicle stimulating and Luteinizing hormone levels were higher. Adolescent girls born prematurely had longer uterus cervix, smaller sizes of endometrial echo, smaller ovarian sizes and fewer follicles in them. Cushing's syndrome in adolescent girls was diagnosed in 3(1.6%) cases from the second group and in 6(1.8%) adolescent girls were diagnosed from the third group.

AMH has not previously been evaluated in adolescent girls and this is the most comprehensive study of circulating AMH levels in Armenia. Random AMH measurement is representative for adolescent girls.

Table 3

The evaluation of ovarian reserve of adolescents

Interpretation of examinations	First group n=170		Second group n=192		Third group n=338	
	n	%	n	%	n	%
AMH						
Normal level (1.5-4.0ng/ml)	130	76.5	115	59.9	224	66.3
High level (over 4.0ng/ml)	-	-	61	31.8	77	22.8
Low normal range (1.0-1.5ng/ml)	35	20.6	10	5.2	26	7.7
Low (0.5-1.0ng/ml)	5	2.9	6	3.1	8	2.4
Very Low (less than 0.5ng/ml)	-	-	-	-	3	0.9
FSH						
Normal level (<10mlU/ml)	130	76.5	115	59.9	242	71.6
High level (13>)	5	2.9	16	8.3	19	5.6
Low level (3.5<)	-	-	61	31.8	77	22.8
LH						
Normal level (2.4-12.6mlU/ml)	130	76.5	115	59.9	242	71.6
High level (12.6>)	-	-	61	31.8	77	22.8
Ovarian Volume, sm³						
Not less than 5 sm ³	130	76.5	85	44.3	224	66.3
From 3 to 5 sm ³	5	2.9	91	47.4	95	28.1
<3 sm ³	-	-	16	8.3	19	5.6
AFC (Antral follicle scout)						
At least 5 in each ovary (10-25)	130	76.5	115	59.9	224	66.3
Not more than 3 on each ovary (<10)	5	2.9	61	31.8	77	22.8
Not more than 2 in each ovary	-	-	16	8.3	19	5.6
Ovarian surgery						
Unilateral	3	1.8	10	5.2	11	3.3
Bilateral	-	-	6	3.1	8	2.4

The data obtained indicate the prevalence of gynecological disease in all three groups. Vulvovaginitis occurs in all age groups, being most common in the second (80.2%) and in the first groups (72.9%). Pelvic inflammatory disease

was most common in the third group 50(14.8%), compared to the first and second 20(10.4%) groups, which significantly complicates the outcome of generative function. According to data obtained, there is a general connection between adolescent pelvic inflammation and negative indicator of reproductive health.

Menstrual cycle disorders were the second common negative indicator of reproductive health [18,20]. Polycystic ovary syndrome (PCOS) is a common condition, present in 61% of adolescents in the second and in 22.8% of adolescents in the third group. Inter-individual variation of AMH levels may be affected by the presence of polycystic ovarian syndrome (31.8% in the second group, 22.8% in the third group) and metabolic syndrome.

AMH was significantly higher in adolescents with polycystic ovary syndrome (PCOS) due to an increased number of antral follicles. AMH plays an inhibitory role in follicular development, inhibitory action on FSH-aromatase, which leads to anovulation and contributes to hyperandrogenism in PCOS [3,19]. This further enhances insulin resistance in these adolescents. Weight management is important in preventing infertility. Adolescents who are overweight or underweight ovulate less regularly compared to adolescents of a healthy weight.

Ovarian reserve was relatively decreased in adolescent girls born with women having complications during pregnancy. But preterm and small for gestational age (SGA) birth did not affect AMH levels in adolescents [15].

A negative impact on the ovarian reserve had ovarian wedge resection, adnexal surgery, electrocoagulation of ovarian tissue after laparoscopic excision of ovarian cysts [16,17]. In the second group the right oophorectomy with the left ovary wedge resection was registered in 10(5.2%) adolescents, bilateral oophorectomy was registered in 6(3.1%) adolescents. In the third group the right oophorectomy with the left ovary wedge resection was registered in 11(3.3%) adolescents, bilateral oophorectomy was registered in 8(2.4%) adolescents. All these adolescents were at a high risk of diminished ovarian reserve.

Conclusions

The study established the normative values for serum AMH and evaluated the normal ovarian reserve of Armenian adolescent girls. Knowledge of low ovarian reserve prediction and its prognostic significance allows to identify risk groups of adolescent girls at an early stage, which allows to correctly determine the reproductive behavior and to master the rest of the reproductive reserve in time to prevent reproductive losses and infertility.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Yerevan State Medical University named after Mkhitar Heratsi. The study was performed

following the Declaration of Helsinki Principles and the written informed consent was given by all participants before enrolment.

Consent for publication

Not applicable in this section since no personally identifiable information is present in our manuscript.

Availability of data and materials

The datasets used in the current study may be available from the corresponding author on reasonable request.

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Competing interests

The authors report that they have no interests which might be perceived as posing a conflict or bias.

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**Уровень антимюллера гормона в сыворотке
крови девочек-подростков**

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В данной работе мы определяли уровень антимюллера (АМГ), фолликулостимулирующего (ФСГ) и лютеинизирующего (ЛГ) гормонов в сыворотке крови среди 700 девочек - подростков до 19 лет. Уровень АМГ в сыворотке крови определялся с использованием метода Elecsys Cobas от Roche Diagnostics, а уровни ФСГ и ЛГ – с помощью иммуноферментного анализа. Диагностическое ультразвуковое исследование малого таза было выполнено с помощью ультразвукового аппарата Toshiba Aplio 500 TUS – A500. В ходе исследования впервые в Армении были установлены нормативные показатели сывороточного АМГ для девочек-подростков и оценен овариальный резерв. Уровень АМГ в сыворотке крови и количество антральных фолликулов в яичниках являются показателями резервной функции яичников. Нормальная резервная функция яичников характеризуется уровнем АМГ в сыворотке крови не менее 2,0 мг/мл, уровнем ФСГ в сыворотке крови не более 10 мЕд/мл, количество антральных фолликулов в каждом яичнике не менее 5 (всего 10-25 антральных фолликулов) и объем яичников не менее 5см³. Показатели АМГ и овариального резерва яичников имеют прогностическое значение для выявления девочек-подростков, входящих в группу риска по абсолютному

и относительно бесплодию, и помогут принять необходимые меры для профилактики бесплодия.

Հակամյուլերյան հորմոնի մակարդակը դեռահաս աղջիկների արյան շիճուկում

Հ.Հ.Բարեղամյան, Ա.Գ. Հարությունյան

Այս աշխատանքում որոշել ենք հակամյուլերյան, ֆոլիկուլո-լիթանիչ և լյուտեինիզացնող հորմոնի մակարդակները մինչև 19 տարեկան 700 դեռահաս աղջիկների արյան շիճուկում: Հակամյուլերյան հորմոնի մակարդակն արյան շիճուկում որոշվել է «Roche Diagnostics» ֆիրմայի «Elecsys Cobas» մեթոդով, իսկ ՖԽՀ-ն և ԼՀ-ն՝ իմունաֆերմենտային եղանակով: Փոքր կոնքի ախտորոշիչ ուլտրաձայնային հետազոտությունը կատարվել է «Թոշիբա Ապլիո 500 » սարքի միջոցով մինչև 19 տարեկան 700 դեռահաս աղջիկների շրջանում:

Առաջին անգամ Հայաստանում հետազոտության ընթացքում հաստատվեցին մինչև 19 տարեկան դեռահաս աղջիկների հակամյուլերյան հորմոնի չափորոշիչները: Գնահատվեց դեռահաս աղջիկների ձվարանային պահուստը: Արյան շիճուկում հակամյուլերյան հորմոնի մակարդակը և ձվարաններում անտրալ ֆոլիկուլների քանակությունը ձվարանների պահուստային ֆունկցիայի ցուցանիշներն են: Ձվարանների նորմալ պահուստային ֆունկցիան դեռահաս աղջիկների համար բնութագրվում է հետևյալ ցուցանիշներով. արյան շիճուկում հակամյուլերյան հորմոնի մակարդակը 2,0մգ/մլ-ից պակաս չէ, ֆոլիկուլոլիթանիչ հորմոնի մակարդակը 10 մՆ/մլ-ից ավել չէ, սոնոգրաֆիկ անտրալ ֆոլիկուլները յուրաքանչյուր ձվարանում առնվազն 5-ն են (ընդհանուր 10-25 անտրալ ֆոլիկուլ), ձվարանների ծավալը 5սմ³-ից պակաս չէ: Հակամյուլերյան հորմոնի և ձվարաններում ֆոլիկուլյար պահուստի ցուցանիշների գնահատումն ունի կանխարգելիչ նշանակություն անպտղության բացարձակ և հարաբերական ռիսկին պատկանող դեռահաս աղջիկների հայտնաբերման համար և թույլ է տալիս իրականացնել անհրաժեշտ միջոցառումներ անպտղության հաղթահարման համար:

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