

ANTIMULLERIAN HORMONE SERUM CONCENTRATIONS IN NORMOOVULATORY AND ANOVULATORY WOMEN OF REPRODUCTIVE AGE

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ABSTRACT

Background

Anti-Mullerian hormone (AMH), produced by growing pre-antral and early antral ovarian follicles, has been shown to be a useful marker for ovarian ageing. Serum AMH concentrations are elevated during reproductive life in anovulatory women, especially in those patients exhibiting polycystic ovaries (PCO) and have been implicated in the pathogenesis of polycystic ovary syndrome (PCOS).

Objectives

The aim of our study is to compare the AMH concentrations in anovulatory women with normoovulatory women of similar age.

Patients and Methods

The study included 70 anovulatory normogonadotrophic women (50 cases with PCOS and 20 cases with Non-PCOS anovulatory women) and 30 cases control group (normal ovulatory women). PCOS patients was diagnosed according to the Rotterdam definition, Serum AMH was measured with a second-generation immunoassay in both groups.

Results

Mean AMH level in anovulatory group was 2.22 ng/ml (0.6-4.10) and for control group was 6.11 (0.10-19.00), there was significant difference in AMH, LH and testosterone level between patients and control group, difference also significant in AMH level between PCOS and non-PCOS subgroup. There was negative correlation between age of the women and AMH level both in control and patient group.

Conclusion

Serum AMH concentrations are elevated in anovulatory, especially in those patients exhibiting PCOS.

Keywords: AMH, Anovulatory, Ovulatory, Reproductive age.

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INTRODUCTION

Anti-Müllerian hormone (AMH), also known as Müllerian-inhibiting substance, is a member of the transforming growth factor- β (TGF β) superfamily, which includes more than 35 structurally related peptides including activins, inhibins, bone morphogenetic proteins (BMPs) and growth differentiation factors. Many of these are involved in the reproductive function of both sexes^(1, 2, 3). The AMH gene is located on chromosome 19 and encodes a 140 kDa dimeric glycoprotein. AMH is synthesised as a pro-hormone, which undergoes cleavage at the site of action to generate a biologically active C-terminal fragment⁽¹⁻⁵⁾.

AMH is strongly expressed in Sertoli cells from the time of testicular differentiation up to puberty and to a much lesser degree in granulosa cells (GCs) from birth up to menopause⁽¹⁾. It has recently been shown that AMH is produced by the growing antral follicles in the human ovary up to the selection stage (4–6 mm), it is clear that the highest expression of AMH is found in preantral and small antral follicles. The latter being those involved in FSH-dependent cyclic recruitment⁽⁶⁾. After selection, the level of expression gradually declines in the mural GCs with the AMH-positive staining becoming localised to the cumulus GCs⁽⁷⁾. Direct measurements of AMH protein production by human GCs and follicular fluid in 2007 confirmed that the highest concentrations were in small antral follicles and became very low or undetectable in follicles ≥ 10 mm^(1, 8).

The cessation of production of AMH from these follicles suggests that this is an important requirement for selection of the dominant follicle. AMH can be used as a marker for ovarian aging, compared to other ovarian reserve markers, only serum AMH level showed a mean longitudinal decline over time. Taken together, these data strongly suggest that serum levels of AMH can be used as a marker of ovarian aging⁽⁹⁾. So many studies done support superiority of AMH over other ovarian hormones as marker of ovarian aging, study done by de Vet *et al.* (2002) reveal that in young normal ovulatory women, early follicular phase hormone measurements at 3-year intervals serum AMH levels decline significantly whereas serum levels of FSH and inhibin B and the number of antral follicles do not change during this interval⁽⁹⁾. The results of de Vet *et al.* (2002) also suggest that changes in serum AMH levels occur relatively early in the sequence of

events associated with ovarian aging⁽⁹⁾. Substantially elevated serum levels of FSH are not found until cycles have already become irregular. Therefore, a marker that already shows a considerable change when cyclicity is still normal would better identify women with declining fertility⁽¹⁰⁾.

The usefulness of serum AMH levels as a measure of the ovarian reserve was recently shown in young women after treatment for childhood cancer. Chemotherapy and radiotherapy treatment have adverse effects on the ovary in particular, resulting in loss of primordial follicles. Serum AMH levels were decreased in these patients, supporting the use of serum AMH levels as an early predictor of the ovarian reserve⁽¹¹⁾.

Several studies have shown that AMH is an excellent marker to determine ovarian responsiveness also in IVF (*in vitro* fertilisation) program. Hormone measurements in the early follicular phase (day 3 of spontaneous cycle), retrospectively or in a group of unselected patients, revealed that AMH levels are lower in patients with poor ovarian response than in women with normal response^(12, 13).

Serum AMH level can also serve as a marker in ovarian pathophysiology, such as polycystic ovary syndrome (PCOS), which characterized by excessive early follicular growth with significantly greater amounts of primary and pre-antral follicles. PCOS is one of the most common endocrine disorders in women of reproductive age⁽¹⁴⁾. It is characterized by anovulation manifested as oligo- or amenorrhea, elevated levels of circulating androgens, and polycystic ovaries as visualized by ultrasound. The diagnosis is based on the presence of at least two of the described characteristics, as defined by the Rotterdam Consensus (2004)⁽¹⁴⁾, likely caused by elevated intraovarian androgens augmenting thecal and granulosa cell growth⁽¹⁵⁾. Furthermore, antral follicle development is arrested at the 4- to 7-mm stage, and dominant follicle selection is disturbed (anovulation). This is proposed to be due to factors including reduced sensitivity to FSH caused by excessive production of local inhibitors of its action such as anti-müllerian hormone (AMH), inhibin or estradiol^(16, 17), or increased LH action due to early LH receptor gain or excessive LH production⁽¹⁸⁾.

AMH concentrations also correlate with other clinical features of PCOS such as cycle duration,

mean ovarian volume, testosterone and androstenedione concentrations, and free androgen index (FAI) ^(19, 20). A rapidly increasing volume of research confirms that circulating concentrations of AMH are elevated in PCOS and that AMH measurement is a specific (92%) and sensitive (67%) marker for the disease ^(21, 22). These initial findings are extremely encouraging and the outcome of more detailed prospective studies is awaited with interest. If confirmed, measurement of the circulating concentration of AMH could provide the most reliable biochemical marker for PCOS. Such work could lead to increasing demand for AMH measurements with a potential decrease in the use of other biochemical markers. However, studies relating AMH not simply to diagnosis of PCOS but also to clinical benefit in terms of leading to improved outcomes are needed to help support the case for AMH measurements.

PATIENTS AND METHODS

This prospective study was conducted in Maternity Teaching Hospital / Erbil in the gynecological outpatient clinic in Erbil city/ Kurdistan Region/North of Iraq. The study included 70 anovulatory women (50 cases with PCOS and 20 cases with non-PCOS anovulatory women) and 30 cases control group ((normal ovulatory women) who participated in a RCT carried out between March 2011 and March 2012. Their age between 15-40 years old in all three study groups and the body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters.

Seventy patients all infertile women attending our fertility clinic between 2011 and 2012 were included in the present study they demonstrate: 1) infertility; 2) oligomenorrhea (interval between periods >35 d) or amenorrhea (absence of vaginal bleeding for at least 6 months); 3) serum FSH concentrations within normal limits (1–10 IU/L) ; positive withdrawal bleeding after progestogen administration in case of amenorrhea; and 5) age between 15 and 40 yr. Clinical investigation, transvaginal ultrasound, and fasting blood was taken on the second or third day of menstrual cycle for hormonal assay (AMH, FSH, LH, testosterone, thyroid function test and prolactin level).

A subgroup of these patients were 50 cases (70-20 = 50 cases with PCOS) , Diagnostic criteria for PCOS included at least two of the following three features: 1) ovulatory disturbance, mainly

oligomenorrhea or amenorrhea, 2) hyperandrogenism (HA) as defined either clinically by hirsutism (modified Ferriman and Gallwey score > 6), or severe acne/seborrhea, and/or biologically by a testosterone serum level greater than 0.7 ng/ml, 3) more than 12 follicles in the 2- to 9-mm range in each ovary at U/S and/or an ovarian volume higher than 10 ml. U/S examination was performed with a 7-MHz transvaginal transducer. US measurements were taken in real time, according to a standardized protocol,

A diagnosis of PCOS was made after excluding other causes of anovulation including prolactin level and thyroid function tests to exclude hyperprolactinaemia and thyroid disorders.

The control group consisted of 30 normo-ovulatory women, Inclusion criteria were regular menstrual cycle (26–30 days), 15-40 years of age, absence of endocrine disorders or any other relevant disease, and no use of medications or oral contraceptives during the 3 months prior to the start of the study.

This study was approved by the scientific committee in Maternity Teaching Hospital in Erbil city north of Iraq, the committee responsible for ethical approval. All patients and controls gave a written consent before their inclusion in this study.

On day 2 or 3 of menstrual cycle, blood samples were collected (both from patients and controls) to measure plasma concentrations of AMH, LH, FSH and testosterone. In PCOS patients, the last menstrual period was either spontaneous or induced by the administration of medroxy progesterone (10 mg/d for 7 days). Any patient with at least one follicle with a diameter greater than 9 mm was excluded from the study in patient group.

Statistical analyses were performed by SPSS version 18.0. Wherever continuous variables were compared at baseline, t-test (in case of two groups), and for comparing categorical variable using chi square test. A p-value of <0.05 was considered statistically significant.

RESULTS

General demographic, clinical characteristics and endocrine data as well as ultrasound findings in control and patient groups are summarized in table 1.

Parameter were compared between patients and control group, In the control group endocrine

parameters were all within the normal range for regularly cycling women; also ultrasound scans revealed normal volume (it was just reported by ultrasonographer as normal ovarian volume) and mean cycle duration for control (ovulatory) group were within normal cycle duration (21-35 days), the mean of BMI was also within normal range (19-25 kg/m²).

There was no statistically significant difference in mean age between patient and control group, with p value of 0.160. The majority of Patients group either had oligomenorrhea or amenorrheic with a mean cycle duration of 45.73 days, which significantly differs from cycle duration of control group which showed a mean of 29.31 days (p<0.00). The mean body mass index was significantly different in patient group, compared with controls with means of 26.9 and 22.11

respectively (P < 0.001). The patients group had elevated LH and testosterone that significantly differs from the control group, but mostly elevated in PCOS group with mean of 7.0 IU/ L and 2.3 nmol/L for LH and testosterone respectively.

Table 2, mean AMH level in patients group 6.11 ng/ml and range of (0.10-19.00) while that for control group was 2.22 ng/ml and range of (0.6-4.10,) there was highly significant difference in mean AMH level between both groups with p value (0.00).

Table 3, the patient group were categorized into PCOS (N=50) and non-PCOS group (N=20), AMH levels were compared between non-PCOS and control group and the difference was significant (p=0.04).

Table 1. Clinical, endocrine, and ultrasound parameters (mean and range) in normoovulatory control subjects compared to normogonadotropic normoestrogenic anovulatory infertile women.

Parameter	Control (N=30)	Patient (PCOS and non-PCOS) (N=70)	P -value
Age (yr)	29.31 (20.6–35.6)	27.5 (19.3–40.8)	0.160
BMI (kg/m ²)	22.13 (18.1–23.7)	26.9 (23–36.3)	< 0.0001
AMH (ng/ml)	2.22 (0.6-4.10)	6.11 (0.10-19.00)	0.00
Cycle duration (d)	29.3 (25-35)	45.73 (25-90)	0.000
FSH (IU/liter)	6.1 (3.3–10.0)	5.5 (2.3–10.0)	<0.01
LH (IU/liter)	3.1 (1.0–6.7)	7.0 (1.1–23.5)	< 0.01
Testosterone (nmol/liter)	1.7(0.3–3.1)	2.3 (0.5–5.5) ¹	< 0.01
Ovarian volume (ml) (per ovary)	Normal	10.3 (3.5–20.8)	

Table 2. The AMH serum levels in normogonadotropic anovulatory infertile women (PCOs) compared with normoovulatory controls.

	Control (N=30)	Patients(PCOS N=50)	p-value
AMH	2.22 (0.6-4.10)	7.07 (1.9-12.1)	0.00

Table 3. The AMH serum levels in normogonadotropic anovulatory infertile women (those without PCO) compared with normoovulatory controls.

	Control (N=30)	Patient(non-PCOS N=20)	p-value
AMH	2.22 (0.6-4.10)	3.66 (0.17-7)	0.04

Table 4, there was a significant negative correlation between age and AMH levels. In the control group, the serum level of AMH decreased with increasing age, with the total 30 cases of control 20 cases has AMH within normal range (p = 0.00).

Table 5, also similarly, a significant negative correlation was found between age and AMH in patients group (PCOS and non-PCOS). Although in PCOS subgroup the AMH level remain higher than non PCOS group, that is mean increasing age associated with decrease in AMH (p= 0.00).

Table 4. Correlation between Serum AMH concentrations vs. age in normoovulatory controls.

Cases (N=30)			AMH			p-value
Age	No.	%	<0.7	0.7-3	>3	
15-25	15	50	0	13	2	0.00
26-35	7	23.33	0	6	1	
36-45	8	26.66	6	1	1	

Table 5. Correlation between Serum AMH concentrations vs. age in anovulatory infertile patients (PCOS and non-PCOS) .

Cases(N=70)			AMH			p-value
Age	NO.	%	<0.7	0.7-3	>3	
15-25	27	38.57	1	6	20	0.00
26-35	27	38.57	1	3	23	
36-45	16	22.85	11	3	2	

DISCUSSION

AMH concentrations may be used as marker in ovarian pathophysiology, such as PCOS. Although the syndrome includes a variety of clinical features, most women with PCOS present with polycystic ovaries, in which the number of pre-antral and small antral follicles is increased, also in women with PCOS, the antral follicle count was well correlated with serum AMH levels. This reflected by the two- to threefold

increase of both markers in women with PCOS⁽²⁰⁾. In addition, serum AMH levels were also correlated with other PCOS features such as cycle duration, ovarian volume and androgen levels^(20, 24). Therefore, it was suggested that AMH may be a marker of the severity of PCOS and thus of ovarian dysfunction.

The present study clearly shows that AMH levels are increased in normogonadotropic anovulatory infertile patients and there is highly significant difference with normogonadotropic ovulatory

women (table 1). The subgroup of patients exhibiting PCOS presents with the highest AMH serum levels. Furthermore, it seems that AMH levels correlate with the extent of ovarian dysfunction in these women, as represented by elevated LH or testosterone levels and an ovarian volume as established on ultrasound

In the Laven *et al.* study⁽²⁰⁾, AMH levels were significantly higher in the PCO group than in non-PCO patients (median, 7.6 µg/liter (range, 0.1–40.0)), compared with controls [median, 2.1 µg/L (0.1–7.4) with $p < 0.001$, Nevertheless, serum levels in non-PCO women were still significantly elevated compared to those in control women (AMH levels were elevated [9.3 µg/L (1.8–40.0)], compared with 22 patients without PCOs [6.4 µg/L (0.1–22.1)] ($P < 0.0001$), which goes with the result of current study which also there was significant difference in AMH level between patients and control also between non-PCOS and control group⁽²⁰⁾.

In the Piltonen T *et al.* study⁽²⁵⁾ forty-four healthy women (aged 21-44 years) and 65 women with previously diagnosed PCOS (aged 16-44 years) participated in the study⁽²⁵⁾, Serum AMH levels were 2- to 3- fold higher in PCOS women than in healthy women, which agrees with the result of the our study with near similar sample size.

In the Park AS *et al.* study⁽²⁶⁾, a prospective study comparing AMH levels in oligomenorrhea (n=24), PCOS (n =153), and normal control adolescents (n = 39), and normal adult women (n = 36). The study was conducted through four tertiary academic medical centers. Basal serum AMH levels were assessed among oligomenorrheic, PCOS, and normal girls, in addition to PCOS and normal women. oligomenorrheic girls had serum AMH levels (5.33 +/- 0.47 ng/ml) that were significantly greater than the normal adolescents (3.05 +/- 0.31 ng/ml) and adults (2.33 +/- 0.22 ng/ml), but similar to values seen in the PCOS adolescents (5.28 +/- 0.26 ng/ml, this result goes with our study regarding higher serum AMH level in anovulatory group including PCOS. while doesn't go with our study regarding the similarity in level of AMH both in PCOS and non- PCOS (tables 2 and 3), because in our study although AMH was elevated in non-PCOS cases but was higher in PCOS group, because in Park AS *et al.* study the level of AMH was significantly higher in oligomenorrheic group regardless of patient are PCOS or not⁽²⁶⁾.

In the Laven *et al.* study⁽²¹⁾ AMH concentrations correlated with features characteristic for polycystic ovary syndrome such as LH concentrations ($r = 0.331$; $P = 0.0001$), testosterone levels ($r = 0.477$, $P = 0.0001$), mean ovarian volume ($r = 0.421$; $P = 0.0001$), and the number of ovarian follicles ($r = 0.308$; $P = 0.0001$), this result goes with the result of our study, in which hormonal profile also correlated with serum level of AMH, was statistically significant (table 1).

In the Laven *et al.* study⁽²¹⁾ AMH levels correlated well with age in patients ($r = -0.248$; $P = 0.002$) as well as in controls ($r = -0.465$; $P = 0.005$), which also goes with the result of current study, as serum AMH level decreased with increasing age.

In conclusion, serum AMH concentrations are elevated in anovulatory women, especially in those patients exhibiting PCOs. AMH may be used as a marker for the extent of the disease (ovulatory disorder specially in polycystic ovarian syndrome cases), it is a new marker which is commonly used in clinical practice in infertility unit specially IVF unit for cases with ovulatory infertility to determine ovarian reserve and degree of respond to IVF treatment, although it's an informative investigation and available in all IVF centers in Kurdistan in in most private laboratory but still it's an expensive test which coasts about 50000 ID.

Further researches are needed in our locality to show more the significance of AMH in infertility.

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