Determination of Anti-Müllerian hormone level in Polycystic Ovary Syndrome, Infertile, and Healthy Iraqi Women

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ABSTRACT

Background: Biochemical markers of ovarian reserve are needed in clinical practice for early detection of ovarian ageing or premature failure, and also to predict poor response to ovarian stimulation in assisted reproduction technologies. Despite limitations to predict pregnancy, anti-Mullerian hormone (AMH) is the most reliable single biochemical marker to predict ovarian response. The finding that AMH can be assessed at any day of menstrual cycle adds a great advantage to this marker over others that require a specific phase for assessment.

Methods: A total of thirty six undiabetic female patients (17 infertile women and 19 with polycystic syndrome) their age ranged from 17-35 years old. Healthy controls (15) matched in sex and age were enrolled in this study for the determination of AMH concentration.

Results: serum AMH concentration is significantly decrease in infertile women(P-value < 0.05) when compared with healthy control, on the other hand there is significantly increase in the level of AMH concentration in women with polycystic ovary as compared with healthy control(P-value < 0.05).

Conclusion: Comparison of female AMH level with respect to average age is useful in fertility assessment, as it provides a guide to ovarian reserve and identifies women that may need to consider either egg freezing or trying for a pregnancy sooner rather than later if their long-term future fertility is poor.

INTRODUCTION

Anti-Müllerian hormone (AMH) is a protein hormone structurally related to inhibin and activin and a member of the transforming growth
Determination of Anti-Müllerian hormone level in Polycystic Ovary Syndrome, Infertile, and Healthy Iraqi Women

Shatha

factor-β (TGF-β) family, it’s a dimeric glycoprotein. It inhibits the development of the Müllerian inhibiting factor (MIF), Müllerian – inhibiting hormone (MIH), and Müllerian – inhibiting substance (MIS). In humans is encoded by the AMH gene on chromosome 19p13.3, it is secreted by Sertoli cells of the testes during embryogenesis of the fetal male. (1, 2)

In mammals, AMH prevents the development of the Müllerian ducts into the uterus and other Müllerian structures. The effect is ipsilateral, that is each testis suppresses Müllerian development only on its own side. This action takes place during the first 8 weeks of gestation. If no hormone is produced from the gonads, the Müllerian ducts automatically develop, while the Wolffian ducts, which are responsible for male reproductive ducts, automatically die. (3)

In healthy females AMH is either just detectable or indetectable in cord blood at birth and demonstrates a marked rise by three months of age; while still detectable it falls until four years of age before rising linearly until eight years of age remaining fairly constant from mid-childhood to early adulthood it does not change significantly during puberty; from 25 years of age AMH declines to undetectable levels at menopause. AMH is expressed by granulose cells of the ovary during the reproductive years, and controls the formation of primary follicles by inhibiting excessive follicular recruitment by FSH. It therefore, has a role in folliculogenesis. (4)

AMH is a substance that is produced by granulose cells in ovarian follicles, it is first made in primary follicles that advance from the primordial follicle stage, at these stages follicles are microscopic and cannot be seen by ultrasound (5). Its level correlate with the number of antral follicles in the ovaries, it has been documented that women with lower AMH have lower number of oocytes when compared with women with higher levels (6).

This hormone levels do not vary with the menstrual cycle and can be measured independently of the day of the menstrual cycle, it can be used for (7): 1-Evaluating fertility potential and ovarian response in vitro fertilization (IVF)-serum AMH levels correlate with the number of early antral follicles. This makes it useful for predicting the ovarian response in an IVF cycle; women with low AMH levels are more likely to be poor ovarian responders. 2-Measuring ovarian aging – diminished ovarian reserve, is signaled by reduced baseline serum AMH concentrations.

The old standard for ovarian reserve testing was the day 3 Follicles stimulating hormone FSH level, however, the FSH level is not as reliable as the AMH level for 3 reasons: The FSH level varies according to the cycle dates, it depends upon the estradiol level (a high
estradiol level will artificially suppress a high abnormal FSH level into
the normal range), and it varies from cycle to cycle, so is not always
reliable or dependable. Thus AMH level is a much better marker for
ovarian reserve, it can be measured on any day of the cycle! This is
why most infertility specialists today use AMH to check ovarian
reserve, rather than the old FSH level. (8)
To assess an individual’s ovarian reserve, early follicular phase serum
levels of FSH, inhibin B and estradiol (E2) have been measured.
Inhibin B and E2 are produced by early antral follicles in response to
FSH, and contribute to the classical feedback loop of the pituitary –
gonadal axis to suppress FSH secretion. With the decline of the follicle
pool, serum level of inhibin B and E2 decrease and subsequently serum
FSH levels rise (9)
Because these factors are part of a feedback system, their serum levels
are not independent of each other.
Furthermore, changes in serum levels of FSH, inhibin B and E2 occur
relatively late in the reproductive aging process (10). So far, assessment
of the number of antral follicles by ultrasonography, the antral follicle
counts (AFC), best predicts the quantitative aspect of ovarian reserve.
However, measurement of the AFC requires an additional transvaginal
ultrasound examination during the early follicular phase. Therefore, a
serum marker that reflects the number of follicles that have made the
transition from the primordial pool into the growing follicle pool, and
that is not controlled by gonadotropins, would benefit both patients and
clinician. (11)
In males, AMH production by the Sertoli cells of the testes remains
high thought childhood in males but declines to low levels during
puberty and adult life, it shown to regulate production of sex hormones
and changing AMH levels (falling in female, rising in males) may be
involved in the onset of puberty in both sexes. (12)
Infertility is the inability to get pregnant after a year of unprotected
intercourse, ovulatory disorders are one of the most common reasons
why women are unable to conceive, and account for 30% of women’s
infertility. (13)
Polycystic ovary syndrome (PCOS), also known as PCOD (polycystic
ovarian disease) is one of the commonest causes of infertility; it is one
of the most common female endocrine disorders. PCOS is a complex,
heterogeneous disorder of uncertain etiology, but there is strong
evidence that it can to a large degree be classified as a genetic disease.
PCOS produces symptoms in approximately 5% to 10% of women of
reproductive age (12–45 years old). It is thought to be one of the
leading causes of female subfertility and the most frequent endocrine
problem in women of reproductive age. (14, 15)
Patients suffering from polycystic ovarian disease (PCOD) have multiple small cysts in their ovaries (the word poly means many). These cysts occur when the regular changes of a normal menstrual cycle are disrupted. The ovary is enlarged; and it produces excessive amounts of androgen and estrogenic hormones. This excess, along with the absence of ovulation, may cause infertility. (16)

Diagnosis of PCOS can be difficult since a specific test can't be performed nor is there a set list of symptoms that doctors can look for. Each woman’s experience of PCOS is unique because no two women have the exact same symptoms. However, a diagnosis is usually made when a woman has irregular or absent periods, in addition to signs of hyperandrogenism without another medical cause. When a woman has infrequent, absent or irregular periods, it is a sign that ovulation may not be occurring.(17,18)

**MATERIALS AND METHODS**

A total of thirty six undiabetic patients were included in this study, they were among patients attending the infertility center in Kamal AL-Samurai hospital during the period between March until May 2012 and their age ranged from 17-35 years old. The patients divided in two groups, the first group consists of seventeen (n=17) infertile women, while the second group consists of nineteen (n=19) with polycystic ovary syndrome (PCOS). Diagnosis was made by specialized doctors in the above mentioned hospital.

Control group consists of fifteen (n=15) healthy women with matched age and normal ovulatory control were selected.

AMH (ELISA) kit was used to determine the serum level of this hormone in the sera of patients and control. (AMH Gen II ELISA, Beckman Coulter, Ref:-A 79765).

**Statistical Analysis:**

Description was done between the three groups (two groups of patients with control) showing mean ± SD, standard error, minimum and maximum values, as shown in table 1.

Multiple comparisons for the level of AMH in the three groups were done (LSD), as shown in table2.

To find the relation between healthy control group and PCO group was shown in table 3, while the relation between the healthy control group and infertile group was shown in table 4.

**RESULTS AND DISCUSSIONS**

For determination the mean of AMH in healthy control group by using the median of these data = 2.4113 (Healthy group), so the normal distribution of this healthy group range between (0.284 -- 3.048) ng/ml.
Levels below 0.284 ng/ml are considered low, and indicate that lower numbers of eggs are within the ovary and decreased fertility. While anything above 5.0 ng/ml is a high value and can indicate PCOS.

The lowest level of serum AMH concentrations were recorded in infertile women, there were significant decrease (P < 0.05) in serum AMH in infertile women (0.4959±0.24777) when compared with healthy control (2.4113±0.69097).

On the other hand there was an increase in the AMH concentration in the sera of women with polycystic ovary syndrome (P< 0.05) in serum of women with PCOS (12.4474±2.60733) when compared with healthy control.

All results were shown in tables: 1, 2, 3, 4, and figures 1 and 2.

The aim of this study was to find the cut-off value of serum AMH in healthy Iraqi women and to assess their usefulness for predicting quantitative and qualitative IVF outcomes in infertile women with and without PCOS; measurement of ovarian reserve is an important part of any infertility evaluation, in addition to identifying those women that may have a very poor chance.

Previously, a number of different hormone blood levels and ultrasound measurements have been used for ovarian reserve testing. These include age, day 3 FSH, inhibin B, antral follicle count, ovarian volume assessment and the clomid challenge test.

Recently, a new hormone marker, AMH has been evaluated as a marker of ovarian reserve, since it is produced only in small ovarian follicles, blood levels of this substance have been used to attempt to measure the size of the pool of growing follicles in women, researchers show that the size of the pool of growing follicles is heavily influenced by the size of the pool of remaining primordial follicles, therefore, AMH blood levels are thought to reflect the size of the remaining egg supply or ovarian reserve.(19)

In women, AMH expression can first be observed in granulosa cells of primary follicles, and expression is strongest in preantral and small antral follicles (≤4 mm). AMH expression disappears in follicles of increasing size and is almost lost in follicles larger than 8 mm.(20)

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.(21) Substantially elevated serum levels of FSH are not found until cycles have already become irregular (10)

Therefore, a marker that already shows a considerable change when cyclicity is still normal would better identify
Determination of Anti-Müllerian hormone level in Polycystic Ovary Syndrome, Infertile, and Healthy Iraqi Women

Shatha

women with declining fertility.

Polycystic ovary syndrome (PCOS), one of the most common endocrine disorders in women of childbearing age, is characterized by a marked increase in pre-antral follicles number. To date, controversial data are available regarding the relationship between the high serum AMH levels and the pre-antral follicles number in PCOS patients. Thus, it is still unknown if the AMH excess in PCOS is secondary to the increase in pre-antral follicles number, or if an intrinsic increased AMH production by the granulosa cells is the cause of follicular arrest in PCOS.(22)

A direct correlation between ovarian antral follicle counts and ovarian volume with hyperinsulinemia was referred in PCOS women. Furthermore, it is unclear if the PCOS-related hyperinsulinemia state could induce the development of antral follicles by increasing the sensitivity of granulosa cells to FSH determining an higher number of follicles and a major ovarian volume(23).

Accumulated data revealed that a lower serum AMH concentration preceding or during assisted reproductive techniques was strongly associated with reduced oocyte yield and low oocyte quality, this might be expected if serum AMH concentrations produced by the small follicles indirectly reflect the remaining follicle pool. Ironically, the opposite appears to be true for successful treatment of infertility in women with polycystic ovary syndrome, in that those who had the highest concentrations of AMH seemed to respond less well.(24,25)

Since PCOS are characterized by an increase in follicle number, this increase has been shown to occur at the earliest stages, and that the increase in AMH concentration is largely due to the increase in production of AMH by each follicle and not just a consequence of an increased in follicle number.

In conclusion, the present study provides that the serum AMH level is an important marker of reproductive aging in women.

In addition, the results of this study provide useful reference date of serum AMH in infertile, PCOS and normo-ovulatory women data of serum AMH ranges in Iraqi women.

Further research of large scale and longitudinal design is necessary to confirm this result. The major problem with determination of AMH level is cost, only few laboratories currently offer the AMH test and the cost is significantly higher than (FSH Level)

Acknowledgment:
I thank all the specialists of infertility center in Kamal AL-Samurai hospital, and I’m grateful to Proff.Dr.Munther Mustafa who helped me in testing and knowledge the AMH, ELISA technique.
Thanks to Prof. Dr. Zaid AL-Madfai (Collage of Medicine, Baghdad University) who had done all the statistic analysis.

Table-1: shows the description of AMH level.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>15</td>
<td>2.4113</td>
<td>.69097</td>
<td>.17841</td>
<td>1.31</td>
<td>3.40</td>
</tr>
<tr>
<td>PCO</td>
<td>19</td>
<td>12.4474</td>
<td>2.60733</td>
<td>.59816</td>
<td>6.90</td>
<td>16.10</td>
</tr>
<tr>
<td>Infertile</td>
<td>17</td>
<td>.4959</td>
<td>.24777</td>
<td>.06009</td>
<td>.09</td>
<td>.82</td>
</tr>
</tbody>
</table>

P< 0.0005

Table-2: shows the multiple comparisons of AMH (LSD)

<table>
<thead>
<tr>
<th>(I) Type</th>
<th>(J) Type</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>PCO</td>
<td>-10.03604*</td>
<td>.56849</td>
<td>.000</td>
</tr>
<tr>
<td>PCO</td>
<td>Infertile</td>
<td>1.91545*</td>
<td>.58306</td>
<td>.002</td>
</tr>
<tr>
<td>Healthy</td>
<td>Infertile</td>
<td>10.03604*</td>
<td>.56849</td>
<td>.000</td>
</tr>
<tr>
<td>Infertile</td>
<td>Healthy</td>
<td>11.95149*</td>
<td>.54949</td>
<td>.000</td>
</tr>
<tr>
<td>PCO</td>
<td>Infertile</td>
<td>-1.91545*</td>
<td>.58306</td>
<td>.002</td>
</tr>
<tr>
<td>Infertile</td>
<td>PCO</td>
<td>-11.95149*</td>
<td>.54949</td>
<td>.000</td>
</tr>
</tbody>
</table>

*. The mean difference is significant at the 0.05 level.
Table-3: shows the cross tabulation type of AMH (normal and high data).

<table>
<thead>
<tr>
<th></th>
<th>Type</th>
<th>Healthy</th>
<th>PCO</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AMH Gp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Count</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within AMH Gp.</td>
<td>100.0%</td>
<td>.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% within Type</td>
<td>80.0%</td>
<td>.0%</td>
<td></td>
<td>35.3%</td>
</tr>
<tr>
<td>High Count</td>
<td>3</td>
<td>19</td>
<td>22</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within AMH Gp.</td>
<td>13.6%</td>
<td>86.4%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Type</td>
<td>20.0%</td>
<td>100.0%</td>
<td></td>
<td>64.7%</td>
</tr>
<tr>
<td><strong>Total Count</strong></td>
<td>15</td>
<td>19</td>
<td>34</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within AMH Gp.</td>
<td>44.1%</td>
<td>55.9%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Type</td>
<td>100.0%</td>
<td>100.0%</td>
<td></td>
<td>100.0%</td>
</tr>
</tbody>
</table>

P< 0.0005
Screening [95% CI]
Sensitivity : 0.80 [0.51; 0.95]
Specificity : 1.00 [0.79; 1.00]
Accuracy : 0.91 [0.75; 0.98]

Table-4: shows the cross tabulation of AMH low data

<table>
<thead>
<tr>
<th></th>
<th>Type</th>
<th>Healthy</th>
<th>Infertile</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AMH Gp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Count</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within AMH Gp.</td>
<td>.0%</td>
<td>100.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% within Type</td>
<td>.0%</td>
<td>23.5%</td>
<td></td>
<td>12.5%</td>
</tr>
<tr>
<td>Normal Count</td>
<td>12</td>
<td>13</td>
<td>25</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within AMH Gp.</td>
<td>48.0%</td>
<td>52.0%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Type</td>
<td>80.0%</td>
<td>76.5%</td>
<td></td>
<td>78.1%</td>
</tr>
<tr>
<td>High Count</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within AMH Gp.</td>
<td>100.0%</td>
<td>.0%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Type</td>
<td>20.0%</td>
<td>.0%</td>
<td></td>
<td>9.4%</td>
</tr>
<tr>
<td><strong>Total Count</strong></td>
<td>15</td>
<td>17</td>
<td>32</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within AMH Gp.</td>
<td>46.9%</td>
<td>53.1%</td>
<td></td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Type</td>
<td>100.0%</td>
<td>100.0%</td>
<td></td>
<td>100.0%</td>
</tr>
</tbody>
</table>

P = 0.031
Screening [95% CI]
Sensitivity : 0.00 [0.01; 0.30]
Specificity : 0.76 [0.50; 0.92]
Accuracy : 0.45 [0.27; 0.64]
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Shatha


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