Anti-Müllerian hormone for prediction of ovarian stimulation response in polycystic ovary syndrome

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Abstract:

Background: Women with polycystic ovarian syndrome (PCOS) are known to have elevated circulating Anti-Müllerian hormone (AMH), which has been found to desensitize ovarian follicles to follicle stimulating hormone (FSH).

Objectives: To determine the predictive value of serum anti-müllerian hormone (AMH) concentration on ovarian response to different stimulation protocols in polycystic ovary syndrome (PCOS).

Patients and Methods: A prospective comparative study was done in Obstetrics and Gynecology department, Qena Faculty of Medicine, South Valley University on 180 infertile women with PCOS attended the infertility outpatient clinic divided into three groups 60 patients each, the 1st group received clomiphene citrate, 2nd group received gonadotrophin, and the 3rd group offered Laparoscopic ovarian drilling, Ovulation and pregnancy rates were measured.

Results: in patients with PCO AMH level with a cutoff value of (3.8 ng/ml), could be used to predict response to CC in obese women, AMH levels of a cutoff value of (4.95 ng/ml), could be used to predict response to Gonadotrophin in women with PCOS, And an AMH level of a cutoff value of (5.74 ng/ml), could be used to predict response to laparoscopic ovarian diathermy in PCOS, there was a statistically significant relation between AMH level and Ovulation and pregnancy rates.

Conclusion: PCOS women with high serum AMH levels seem to be resistant to ovulation induction by clomiphen citrate, gonadotrophin and laparscopic ovarian drilling. Pretreatment measurement of serum AMH concentrations may therefore be a valuable predictor of success and may help in determining the starting dose.

Keywords: Anti-Müllerian hormone, ovarian stimulation response, polycystic ovary syndrome**.**

Introduction:

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, and the most common cause of anovulatory infertility***(Azziz et al., 2006).***

The Rotterdam criteria for PCOS, issued in 2003 by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), includedoligoovulation and/or anovulation, hyperandrogenemia and/orhyperandrogenism (clinical signs or elevated androgen levels), and PCOMon ultrasound evaluation. A diagnosis of PCOS requires the presence of at least two of the three features, afterthe exclusion of other androgen excess disorders**(The Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004;*Azzizet al., 2006*).**

Biochemical signs of hyperandrogenism (HA) strongly correlated to both Anti-Müllerian hormone (AMH) level, and antral follicle count (AFC)(***Chenet al., 2008***).So one can be used in place of the other since they strongly correlate to each other***(Nardoet al., 2009).***

Serum AMH levels are proportional to the number of developing antral follicles in the ovaries and may represent both the quantity and quality of the ovarian follicle pool ***(La Marca et al., 2006).***

Serum AMH levels are the main marker of the ovarian follicular reserve used in human assisted reproduction to evaluate the ovarian stimulationprotocols***(Nestler, 2008).***
AMH level decreases during the reproductive time and becomes undetectable at the time of menopause. On the other hand, an increased level of AMH can be observed in females suffering from PCOS, which indicates the presence of a larger number of antral follicles. This hormone is a practical indicator of ovarian reserve and can serve as a predictor of ovarian response in cycles of in vitro fertilization (IVF)***(Gupta et al., 2019).***

Objectives:
To determine the predictive value of serum AMHlevel on the ovarian response to different stimulation protocols in patients with PCOS.

Materials and Methods:
This was a prospective comparative study conducted on180 infertile women with PCOS who attended the infertility outpatient clinic at Obstetrics and Gynecology Department, Qena Faculty of Medicine, South Valley University,and fulfilled the inclusion and exclusion criteria.
The study included patients with ages between 18 and 39 years, anovulatory infertility and a diagnosis of PCOS based on Rotterdam consensus criteria (two of three criteria: oligo/ anovulation, hyperandrogenemia, and sonographic appearance of polycystic ovaries);with no other causes of infertility, e.g. (tubal factor, male factor) voluntary participation with informed consent.
The study excluded patients who lost the follow-up or with incomplete data, women with other causes of anovulation such as thyroid dysfunction and hyperprolactinaemia. Patients with marked hyper-androgenaemia) either clinical or biochemical; based on the presence of hirsutism (modified Ferriman and Gallwey score > 6) or severe and diffuse acne/ seborrhea. Or total testosterone serum level is > 0.76 ng/ml (normal range 0.14–0.76 ng/ml) Other PCOS like syndromes (late-onset congenital adrenal hyperplasia, androgen producing tumors, Cushing's syndrome), hyper-prolactinemia and thyroid abnormalities: gross ovarian pathology either diagnosed by ultrasound or by laparoscopy, any uterine pathology diagnosed by ultrasound or by endoscopy suspected to cause infertility.Other causes of infertility, even if diagnosed during laparoscopy such as tubal pathology and pelvic endometriosis or adhesions. Previous tubal or ovarian surgery and contraindications to laparoscopy and general anesthesia
All patients were subjected to:
A full detailed history; physical examination; height and weight were measured followed by calculation of BMI = [wt/(ht)^2]. Abdominal examination, Local examinationUltrasound: Trans-abdominal and/or trans-vaginal ultrasound, to exclude patients with ovarian masses or pelvi-abdominal masses.Transvaginal ultrasound assessment of AFC and ovarian volume, carried out on cycle day 3 using an ultrasound machine with a 7.5 MHz transvaginal after the localization of both ovaries, round or oval sonolucent structures in the ovaries were regarded as follicles. Follicles measuring less than 10 mm in diameter were counted in both ovaries to determine the antral follicle cohort. The total number of follicles in both ovaries was used as the AFC.Ovarian volume was calculated by the formula (Volume = 0.526 × Length × height × width).Serum samples for hormonal assays were taken on days 2–3 of the spontaneous menstrual cycles before treatment for measurements of serum FSH, LH, E2, AMH, TT.Patients were divided into 3 groups. Each group included 60 patients.

- **First group**: Patients received Clomifene Citrate(CC) as per standard protocol, i.e., starting with a daily dose of 50 mg for 5 days the early follicular phase (day 2 or 3) of spontaneous menstrual period or progestogen induced withdrawal bleeding. They were advised to have timed intercourse. Patients were monitored for ovulation (main outcome measure) by follicular tracking (with trans-vaginal ultrasound). In women not responding to 50 mg CC in the first cycle, the dose was increased 100 mg then 150 mg if necessary in the subsequent cycles. Responders were followed up until the pregnancy was achieved as determined by a urinary human chorionic gonadotropin test (maximum three cycles of treatment).
- **Second group:** Patients received gonadotropins as minimal stimulation protocol (low dose protocol), i.e., starting

with a daily dose of 37.5 -75 IU (Merional) for 7-14 days in the early follicular phase (day 2 or 3) of the spontaneous menstrual period or progestogen induced withdrawal bleeding. They were advised to have timed intercourse.

- **Third group:** Patients offered laparoscopic ovarian surgery, 4 punctures to the ovary. Patients were monitored for ovulation by follicular tracking (with transvaginal ultrasound), responders were followed up until the pregnancy was achieved as determined by a urinary human chorionic gonadotropin test (maximum three cycles of treatment).
- **Main Outcome Measures:** Ovulation rate and pregnancy rates.

Ethical considerations:

The study protocol was approved by the local Ethical Committee of Qena Faculty of Medicine, South Valley University. Informed written consent was taken from all patients and their husbands before starting the study and every patient had the right to leave the study at any time.

Blood sampling:

10 mm venous blood samples were collected from all participants on cycle day 2/3 (of a normal or induced bleed) before recruitment into the study to measure baseline serum concentrations of AMH, FSH, LH, Prolactin, and Testosterone. All Hormonal assays were done according to the supplier's instructions by Electrochemiluminescence (ECL) technology using an automatic clinical platform assay instrument (**Cobas e**411 analyzer -Roche Diagnostics GmbH-Mannheim-Germany) using Roche's standard protocol. The intraassay and interassay coefficients of variation of all the assays were all less than 10%.

- AMH: sandwich assay, measuring range 0.01-23 ng/ml (0.071 - 164.2 pmol/L)
- LH: sandwich immunoassay, measuring range 0.100–200 mIU/ml
- FSH: sandwich immunoassay, measuring range 0.100-200 mIU/ml
- Prolactin: sandwich immunoassay, measuring range 1.00–10,000 µIU/ml, Expected values women (non-pregnant): 102–496 µIU/ml (2.5th – 97.5th percentile, n = 198)
- Testosterone: competitive immunoassay with analyte liberation, Measuring range 0.025–15.0 ng/mL (0.087–52.0 nmol/L), Expected values Women 20 – 49 years of age 0.084 – 0.481 ng/ml

Statistical analysis:

Statistical Package for Social Sciences (SPSS) software program (version 20) (SPSS Inc., Chicago, IL-USA), was used for data analysis. Shapiro-Wilk test was conducted to assess normality. A qualitative variable was recorded as frequencies and percentages and compared by chi-square test. Depending on the normality of distribution of variables, the comparisons between groups to determine the significance of inter-group differences performed using either Student t-tests (normal distribution of variables) or the Mann-Whitney U-test (non-normal distribution). The quantitative measure was presented as a mean (M) ± standard deviation (SD).

Results:

This was a prospective comparative study involving 180 PCOS cases; 45% of cases were overweight and 35% were obese. 71.67% had primary infertility. 72.78% had acne and/or hirsutism, demographic, clinical, and laboratory data, Table 1.

Table 1. Demographic, clinical and laboratory data in PCOS cases

All PCOS cases data	**NO = 180**
Age (years) **Mean ±SD** **Median (Range)**	 28.4±4.83 28(18-38)
BMI (kg/m2) **Mean ±SD** **Median (Range)**	 28.05±4.22 27(20-39)
Obesity (BMI)	No (%)

(18.5-24.9) normal **(25-29.9) overweight** **(≥ 30) obese**	36(20%) 81(45%) 63(35%)
Duration of infertility (years) **Mean ±SD** **Median (Range)**	 6.7±2.92 7(2-12)
Type of infertility • **1ry infertility** • **2ry infertility**	No (%) 129(71.67%) 51(28.33%)
Hirsutism/acne • **No** • **Yes**	No (%) 49(27.22%) 131(72.78%)
Antral follicular count **Mean ±SD** **Median (Range)**	 12.27±4.32 11(7-24)
Ovarian volume (cm³) **Mean ±SD** **Median (Range)**	 12.28±6.85 10(4-33)
FSH (mIU/ml) **Mean ±SD** **Median (Range)**	 5.66±1.63 5.8(2.7-9.2)
LH (mlU/ml) **Mean ±SD** **Median (Range)**	 7.28±3.57 6.9(1.9-14)
E2 (pg/ml) **Mean ±SD** **Median (Range)**	 38.36±10.86 39(16-65)
AMH (ng/ml) **Mean ±SD** **Median (Range)**	 5.24±2.47 5(2-12)
Testosterone (ng/ml) **Mean ±SD** **Median (Range)**	 0.75±0.27 0.7(0.4-1.5)

In PCOS cases (group I), there was a statistically significant increase in the mean serum level of AMH serum level that necessitates increasing the dose of CC, Table 2.

Table 2. CC doses in group I in relation to AMH level

Group I: CC (no=60)	**AMH (ng/ml)**	**ANOVA**	***p-value***
50mg (no=20)	2.87±0.92	8.291	**<0.001****
100mg (no=28)	4.08±1.30		
150mg (no=12)	5.79±1.85		

*significant

The accuracy and the diagnostic performance to define the best cutoff value of AMH serum level to discriminate ovulation responders and non-responders to ovulation induction by three different methods were compared with the area under the receiver operating curves (AUROC) and the probability of a true positive (sensitivity) and a true negative (specificity), positive predictive value (PPV), negative predictive value (NPV), were calculated, table 3.

Group I CC: AMH cutoff of 3.8ng/ml was the most significant predictor of ovulation induction, with the highest AUC 0.833, a sensitivity of 84% specificity of 80% positive predictive value of 95.5%, the negative predictive value of 50% with a diagnostic accuracy of 83.3%.

Group II Gonadotropins: AMH cutoff of 4.95ng/ml, with a sensitivity of 70.8% specificity of 58.3% positive predictive value of 87.2%, the negative predictive value of 33.3% with a diagnostic accuracy of 68.3%.

Group III Drilling: AMH cutoff was 5.74ng/ml, with a sensitivity of 67.4% specificity of 50% positive predictive value of 81.6%, the negative predictive value of 31.8% with a diagnostic accuracy of 63.3%.

Table 3. Diagnostic performance of AMH serum cutoff level (ng/ml) to discriminate ovulation responders and non-responders

Groups	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
CC	3.8	84.0%	80.0%	95.5%	50.0%	83.3%	0.833
Gonadotropins	4.95	70.8%	58.3%	87.2%	33.3%	68.3%	0.683
Drilling	5.74	67.4%	50.0%	81.6%	31.8%	63.3%	0.633

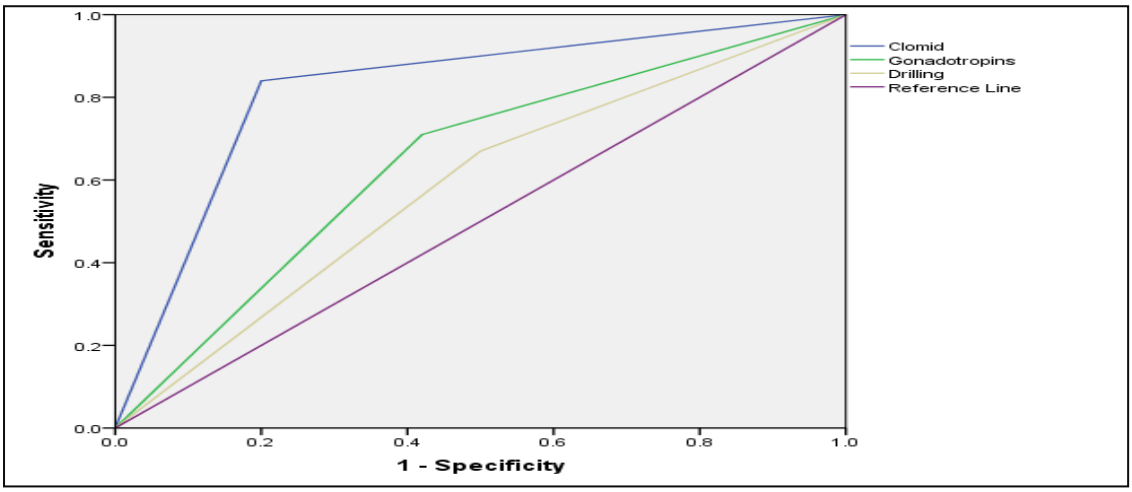

Figure 1. ROC curve of AMH cutoff level for the prediction of ovulation in response to CC, Gonadotrophins and drilling

The ovulation/patient in PCOS cases; showed a statistically significant relation with AMH serum levels in each group, with no significant difference in the rate of pregnancy/patients in PCOS cases, Table 4.

Table 4.Pregnancy and ovulation rates per patient in relation to AMH serum levels in response to different ovulation induction methods in PCOS groups

AMH level	Ovulation/patient	Pregnancy/patient
Group I: CC		
AMH <3.8 ng/ml (n=44)	42(95%)	22(50%)
AMH ≥3.8 ng/ml (n=16)	8(50%)	4(25%)
p-value	**<0.001***	**>0.05**
Group II: Gonadotrophins		
AMH <4.95 ng/ml (n=39)	34(87%)	10(26%)
AMH ≥4.95 ng/ml (n=21)	14(67%)	2(10%)
p-value	**<0.05***	**>0.05**
Group III: drilling		
AMH <5.74 ng/ml (n=38)	31(82%)	6(16%)
AMH ≥5.74 ng/ml (n=22)	15(68%)	2(9%)
p-value	**<0.05***	**>0.05**

The ovulation/cycle in PCOS cases; showed a statistically significant relation with AMH serum levels in each group, with no significant difference in the rate of pregnancy/cycle in PCOS cases, Ttable5.

Table 5. Pregnancy and ovulation rates per cycle in each group

AMH level	Ovulation/cycle	Pregnancy/cycle
Group I: CC		
AMH <3.8 ng/ml	81%	25%
AMH ≥3.8 ng/ml	36%	15%
P-value	**<0.001***	**>0.05**
Group II: Gonadotropins		
AMH <4.95 ng/ml	74%	20%
AMH ≥4.95 ng/ml	41%	12%
P-value	**<0.05***	**>0.05**
Group III: drilling		
AMH <5.74 ng/ml	67%	16%
AMH ≥5.74 ng/ml	47%	10%
P-value	**<0.05***	**>0.05**

*significant

Discussion:

In this study, we have evaluated the impact of estimation ofAMH on the outcome of ovarian stimulation in 180 women having anovulatory PCOS patients dividing into three groups, we found that the circulating AMH levels were negatively correlated with ovarian response to different stimulation protocol.

In this study, there was a significant association between the AMH leveland other factors, including age, BMI, LH, testosterone, type, and duration of infertility.

In contrast with the Greenwood cohort study of 640 PCOS patients, there was no significant relationship between the AMH leveland other factors, including age, BMI and FSH, and LH level. However, infertility duration was the only factor significantly associated with the AMH level. This could be due to a high level of AMH in women with severe PCOS, which led to more resistance to treatment ***(Greenwood et al., 2018).***

In a previous study, more than 50% of PCO patients, were obese, which illustrates that obesity is a risk factor for PCOS. About 50% of PCOS women are overweight or obese and the history of the weight gain usually precedes the onset of oligomenorrhea and hyper-androgenism, suggesting a pathogenic role of obesity in the subsequent development of the syndrome ***(Darwish et al., 2017)***.

In our study, approximately 45% of all PCOS women having overweight and 35% were obese and with a history of weight gain frequently precedes the onset of oligomenorrhea and hyper-androgenism.

In this study, approximately 73% of all PCOS women had hirsutism/acne, (70%) in group I, (73%) in group II and (75%) in group III of PCOS infertile patients.

In this study, we detected a significant difference between groups according to the AFC, which may be a surrogate for the AMH level.

We found a cutoff level of serum AMH concentration (3.8 ng/ml) for group 1 (CC), (4.95 ng/ml) for group 2 (gonadotrophin) and (5.74 ng/ml) for group 3 (LOD), above which the chances of good ovarian response were markedly reduced from 95% (in women with lower AMH) to 50% in 1st group, 87% (in women with lower AMH) to (67%) in group 2 (gonadotrophin), and from (82%) to (68%) in group 3 (LOD).

Our results provide an addition to the available evidence supporting the correlation between high AMH levels and poor ovarian response to treatment,

In contrast, El-Halawaty found that AMH levelsat a cutoff value of (1.2 ng/ml) could be used to predict response to CC in obese women

with PCOS. They also noted that the FSH concentration was not significantly different between women who responded to CC and those who did not ***(El-Halawaty et al., 2007)***.

In our study, we found that AMH level, at a cutoff value of (3.8 ng/ml), could be used to predict response to CC in obese women with PCOS, Also we found that AMH level, a cutoff value of (4.95 ng/ml), could be used to predict response to Gonadotrophin in women with PCOS, And found that AMH level, a cutoff value of (5.74 ng/ml), could be used to predict response to laparoscopic ovarian diathermy in PCOS, but ***(Amer et al., 2009),***concluded that pretreatment circulating AMH level, at a cutoff value of 7.7 ng/ml, seems to be a good judge of the ovarian response to laparoscopic ovarian diathermy in PCOS with 78% sensitivity and 76% specificity,in contrast to our study which was 67% sensitivity and 50% specificity to AMH cutoff (5.74).

Previous study was conducted for 60 PCO patients who have shown significant differences in AMH values. The cutoff value was 3.4 ng/ml. This discrepancy may be attributed to some extent the smaller number of patients in the previous study ***(Mahran et al., 2013).***

Ovulation and pregnancy rates were significantly higher (95% and 46%) in patients with low AMH (< 3.4 ng/ml) versus women with AMH ≥3.4 (48% and 19%). It may be postulated that in those women with anovulatory PCOS who have very high granulosa cell production of AMH, as reflected by profoundly elevated serum AMH levels, the inhibitory actions of AMH on folliculogenesis cannot be overcome by weight loss treatment or gentle ovulation induction regimens***(Shahin et al., 2019).***

In this study ovulation and pregnancy rates were significantly higher (97% and 50%) in patients with low AMH (< 3.8 ng/ml) versus women with AMH ≥3.8 (50% and 25%),in group 1 CC,in group 2 gonadotrophins ovulation and pregnancy rates were (87% and 26%) in patients with low AMH (< 4.95 ng/ml) versus women with AMH ≥4.95 (67% and 10%), and in group 3 (LOD) ovulation and pregnancy rates were (82% and 16%) in patients with low AMH (<5.74 ng/ml) versus women with AMH (≥5.740 (68% and 9%).

In previous study, of 60 women with PCOS they found that day 3 serum AMH concentration ≥3.2 ng/ml was a predictor of IR and clinical pregnancy rate with 72.1% and 75.6% sensitivity and 72.7% and 77.3% specificity, respectively ***(Kaya et al., 2010).***

In contrast to this study, we study 180 PCOS patients divided to three groups 60 patients each, ovulation and pregnancy rate were 95%, 50% in 1st group (CC) below the AMH cutoff (3.8), and 50%, 25% in the same group above the AMH cutoff (3.8), in 2nd group (gonadotrophins), ovulation and pregnancy rate were 87%, 26% below the AMH cutoff (4.95), and 67%, 10% in the same group above the AMH cutoff (4.95), in 3rd group (Drilling) ovulation and pregnancy rate were 82%, 16% below the AMH cutoff (5.74), and 68%, 9% in the same group above the AMH cutoff (5.74).

In previous study of 113 PCOS patients, they found that AMH ≥8.5 ng/ml was associated with no ovulation after LOD (sensitivity 74% and specificity 69%, Also LH ≥15.2 IU/l was associated with no ovulation after LOD (and sensitivity of 71% and specificity of 73%) ***(Rezk et al., 2016).***

In another study, included anovulatory women with PCOS undergoing LOD (no = 29) or receiving CC (n= 18). Plasma AMH levels were measured before and 1 week after treatment. Further measurements of AMH were made at 3- and 6-month follow-up. AMH was found to be a useful predictor of no ovulation after LOD Using a cutoff of 7.7 ng/ml, AMH had a sensitivity of 78% and a specificity of 76% in the prediction of no ovulation after LOD ***(Hashim et al., 2015).***

In this study, we use AMH at cutoff (5.74ng/ml), and found a useful predictor of ovulation before LOD with a sensitivity of 67% and a specificity of 50% in the prediction of ovulation,and above this cutoff the ovulation and pregnancy rate decreased to 68%, 9% respectively.

Based on the data presented in this study, we believe that serum AMH seems to be a good predictor of the ovarian response to different stimulation protocol. This could help with counseling women with PCOS regarding the chance of success with treatment.

Conclusion:
In conclusion, PCOS women with high serum AMH levels seem to be resistant to ovulation induction by clomiphen citrate, gonadotrophinand laparscopic ovarian drilling. Pretreatment measurement of serum AMH concentrations may therefore be a valuable predictor of success and may help in determining the starting dose.

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