Archive of Obstetrics, Gynecology and Reproductive Medicine
AOGRM, 3(1): 61-65
www.scitcentral.com

ISSN: 2640-2297

Original Research Article: Open Access

Anti-Müllerian Hormone as a Predictor of Ovarian Response to Clomiphene Citrate in Women with Polycystic Ovary Syndrome

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Received November 05, 2019; Revised December 21, 2019; Accepted December 23, 2019

ABSTRACT

Background: Anti-Müllerian (AMH) hormone is a member of the TGF-b superfamily of growth factors produced by granulosa cells of pre- and small-antral follicles. The 2 to 3 fold increase in the number of growing follicles in the ovary from PCOS women is reflected by the rise in serum concentration of AMH and thus. This hormone may be a useful marker of PCOS. Also it is expected that it may be related to the clinical and biochemical variables and the response to induction of ovulation. This study was conducted to detect the predictive role of AMH in women with PCOS undergoing induction of ovulation using clomiphene citrate.

Methods: This was a case-control study carried out at the outpatient clinic of obstetrics and gynecology — Suez Canal University Hospitals. Sera from patients with PCOS (n=22) and control women (n=22) were used for ELISA measurement of AMH (AMH-EIA. Beckman Coulter) with sensitivity of 0.7 pmol/L. Then PCOS group was subjected to induction of ovulation with clomiphene citrate.

Results: We found a profoundly high AMH level among the PCOS group compared to the control (50.18 ± 12.44 and 31.68 ± 5.69 pmol/L, respectively, P value=0.0001). We observed a significant positive relation of AMH level with MFGS (r=0.801, P value=0.0003), AFC (r=0.58, P value 0.0001), 2-5 mm pool (r=0.637, P=0.0001), ovarian volume (r=0.744, P value=0.002), LH (r=0.855, P value=0.0001) and total testosterone (r=0.883, P value=0.0001). The only significant negative relation was with FSH level (r=-0.726, P value=0.0001). Response to induction of ovulation was better among PCOS group with lower AMH level.

Conclusion: Measurement of serum AMH may be used as a valuable marker for PCOS to confirm the diagnosis and evaluate the extent of follicular dysfunction and can still be used as a marker for response to induction.

Keywords: Anti-mullerian hormone, Polycystic ovary syndrome, Induction, Clomiphene citrate, Prediction

INTRODUCTION

Infertility affects about 15% of couples [1]. Anovulation, tubal adhesions as well as male factor infertility are the leading causes of infertility, with polycystic ovary syndrome (PCOS) the most encountered cause in anovulation [2], with a prevalence of 5-10% [3]. Clomiphene citrate (CC) was the first line of management in women with PCOS for the induction of ovulation [4]. Clomiphene citrate has fewer side effects, an acceptable success rate, and a lower cost when compared to drugs used in superovulation [5]. Up till now. Anti-Mullerian Hormone (AMH) plays a vital role in the evaluation of ovarian reserve [6]. AMH is a member of the transforming growth factor family. It is secreted from the granulosa cells of the pre-antral and antral follicles. Its concentration represents a reflection of the quality of the follicular pool, making it a useful marker for ovarian reserve [7]. It is increased 2-3 folds in women with PCOS than women with normal ovaries. It was suggested that AMH decreased follicular sensitivity to FSH and reduced the production of estradiol [8]. It was found that serum AMH levels were correlated with the number of oocytes developed

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Citation: Ibrahim ZM, Gharieb WF, Elnahas KM, Metawei MA, Shora HA, et al. (2020) Anti-Müllerian Hormone as a Predictor of Ovarian Response to Clomiphene Citrate in Women with Polycystic Ovary Syndrome. Arch Obstet Gynecol Reprod Med, 3(1): 61-65.

during ovulation induction using gonadotropins. Serum AMH levels were critical in the prediction of the number of oocytes at controlled hyperstimulation in *in vitro* fertilization (IVF) cycles [7]. Also, its predictive role was evaluated in women with unexplained infertility undergoing induction of ovulation using CC [9]. The current study evaluated its predictive role in women with PCOS undergoing conventional induction of ovulation using CC. This would be helpful to avoid unfruitful treatment in women deemed to have poor response to CC.

PATIENTS AND METHODS

This study was approved by the research ethics committee board of Suez Canal University. In which we have enrolled a total of 44 women presented to Suez Canal university Gynecology clinic. We have further stratified them into two groups. The case group included those with polycystic ovary syndrome defined according to revised Rotterdam criteria. 2003 with at least two of the following three criteria were met: a) Oligomenorrhea (less than 8 spontaneous menstrual cycles per year for at least 3 years before enrollment) or amenorrhea or persistent anovulation; b) biochemical hyperandrogenemia (serum total testosterone level ≥ 0.8 ng/ml) and/or modified Ferriman Gallawy score ≥ eight and/or acne; and c) polycystic ovaries (≥ 12 follicles in the 2-9 mm range and/or an ovarian volume ≥ 10 ml per ovary by vaginal ultrasound) [10].

The diagnosis of PCOS was retained after excluding hyperprolactinemia, thyroid dysfunction, Cushing's syndrome, an adrenal tumor, an ovarian tumor, current or previous use of oral contraceptives within six months of enrollment, or the use of medications known to affect the hypothalamic-pituitary ovarian axis such as anti-androgens, ovulation induction agent, anti-diabetic drugs, anti-obesity medications or glucocorticoids.

The control group was composed of 22 healthy women consulting in the Obstetrics and Gynecology Department of Suez Canal University hospital with regular menses, proven fertility (at least one child) and with no clinical features of PCOS and who have consented for enrollment into the study. Control of the confounding factors of age and BMI was achieved by keeping in mind limitation of both during inclusion of each group and also, by exclusion of other conditions simulating PCOS as previously mentioned.

Transvaginal US was done to confirm the diagnosis of PCOS and to detect the antral follicle count (AFC), which was further classified into 2-5 mm and 6-9 mm follicular pool.

The hormonal assay included total serum testosterone. Serum prolactin. Midluteal serum progesterone. Serum FSH and LH were estimated for the PCOS group.

AMH assay

Serum AMH was measured in duplicate using ultra-sensitive ELISA (AMH-EIA, Beckman Coulter, Villepinte, France). The intra-assay coefficient of variation was 12%. The detection limit was 0.7 pmol/L. All samples were taken between day 2 and day 7 of the cycle and stored at -20°C.

The study group had induction of ovulation using Clomiphene citrate 50 mg tablets (Clomid® 50 mg) applied twice daily for five days starting from day 2 of natural or progesterone-induced cycle. In the absence of conception, treatment was continued for three successive periods. In cases of failed response to clomiphene citrate the drug dosage was increased by 50 mg till ovulation was achieved or the three cycles are completed. Folliculometry was done through transvaginal ultrasound on days 9, 11 and 13 of the period. Successful induction was deemed when a dominant follicle reached 18-25 mm.

RESULTS

Both groups were matched regarding their age (p-value 0.52). They differed significantly in their BMI, ovarian volume and AFC (p-value 0.03, 0.0001 and 0.0001, respectively). Of the PCOS group, 86.3% had a BMI>25. hyperandrogenism, anovulation and menstrual irregularity were prominent in 40.9%, 100% and 81.8% of women with PCOS, respectively **(Table 1)**.

Table 1. Patients' demographic data.

Variables	PCO group (N=22)	Control group (N=22)	P value
Age	25.95 ± 2.02	25.5 ± 3	0.52
BMI	27.54 ± 2.01	23.81 ± 3.6	0.03
Mean ovarian volume	11.25 ± 2.4	6.64 ± 0.94	0.0001
Mean AFC	12.5 ± 2.9	6.5 ± 1.11	0.0001
AMH	50.18 ± 12.44	31.68 ± 5.69	0.0001

Serum AMH levels correlated significantly with all patients' parameters in the study group but for BMI (p-value 0.52). However, AMH levels associated significantly with BMI, AFC and the ovarian volume in the control group (p-value 0.05, 0.0001 and 0.002, respectively) **(Table 2)**.

Table 2. Correlation between serum AMH levels and PCOS patients' characteristics.

PCOS group		
	r	**P value**
BMI (kg/m^2)	0.062	0.52
MFGS	0.801	0.0003
AFC	0.58	0.0001
2-5 mm POOL	0.637	0.0001
6-9 mm POOL	0.136	0.35
Ovarian Volume (ml)	0.744	0.002
FSH (IU/L)	-0.726	0.0001
LH (IU/L)	0.855	0.0001
Total Testosterone (ng/ml)	0.883	0.0001

The mean AMH levels were significantly lower in responders to CC induction as well as those who achieved conception (p-value 0.0001 and 0.007, respectively) **(Table 3)**.

Table 3. AMH level and response to induction of ovulation.

The area under the curve	78%
Standard error	0.072
p-value	0.001 (sig)
95% CI	0.644-0.924

The diagnostic performance of AMH was assessed using the ROC curve. The AUC of serum AMH assay 78% with 95% CI7 (0.644-0.924). With a cut-off value of 40.5 pmol/L, the serum AMH level had a specificity of 78% and a sensitivity of 73% **(Table 4 and Figure 1)**.

Table 4. Analysis of the ROC curve.

	Mean AMH level (Pmol/L)	**P value**
Non-responders	59.1 ± 11.9	0.0001
Responders	42.75 ± 6.9	
Conception	35.75 ± 3.3	0.007
No conception	53.4 ± 11.4	

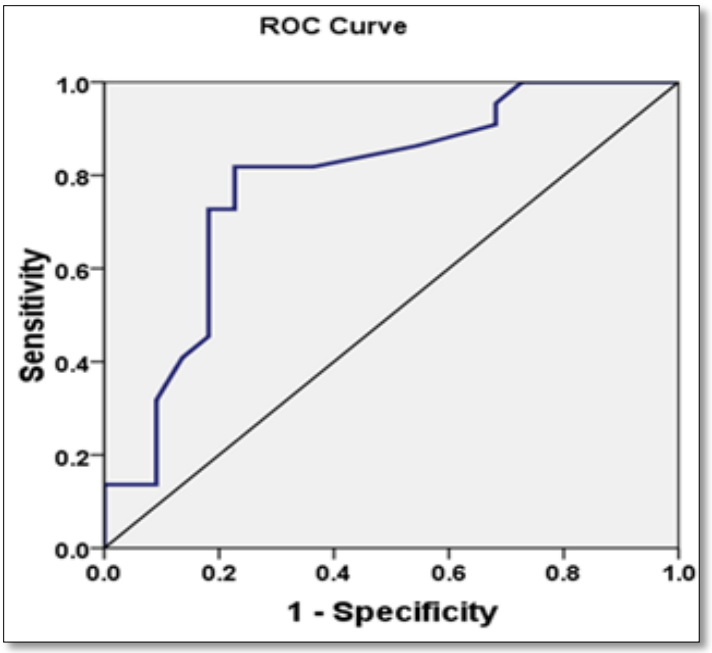

Figure 1. ROC (receiver operator curve).

DISCUSSION

This study demonstrated the clinical and laboratory characteristics of patients with PCOS. There was a significant increase in their BMI than the control group (27.54 ± 2.01, 23.81 ± 3.6, respectively with a p-value of 0.03). Hyperandrogenism was reported in 40.9% of patients. All the patients showed evidence of anovulation. Menstrual irregulatory was predominant in those patients (81.8%). Serum AMH levels were significantly higher in women with PCOS (50.18 ± 12.44, 31.68 ± 5.69, respectively and a p-value of 0.0001). It was documented that serum AMH levels are 2-3 times higher in women with PCOS than normal women [11].

Serum AMH levels correlated significantly with all patients' parameters in the study group but for BMI (p-value 0.52). This was reported in a previous study [12,13]. However, AMH levels correlated significantly with BMI, AFC and the ovarian volume in the control group (p-value 0.05, 0.0001 and 0.002, respectively).

The mean AMH levels were significantly lower in responders to CC induction as well as those who achieved conception (p-value 0.0001 and 0.007, respectively). This was similar to previously reported results by Xi et al. [12] (5.34 ± 1.97 vs.7.81 ± 3.49, P<0.001 for responders and none responders) and (4.81 ± 2.06 vs. 6.89 ± 2.95 ng/ml, P<0.01 for patients who conceived and those who did not) as well as others [14-16]. Generally, the more dominant the follicle, the more decrease in AMH would be achieved [13]. They reported a predictive role of serum AMH for the ovarian response in univariate and multivariate analysis [12] and in univariate analysis only in another study [14].

The diagnostic performance of AMH was assessed using the ROC curve. The AUC of serum AMH assay 78% with 95% CI7 (0.644-0.924). With a cut-off value of 40.5 pmol/L, serum AMH level had a specificity of 78% and a sensitivity of 73%. This agreed with a previously reported result in which a serum AMH concentration of 7.77 ng/ml had a sensitivity and a specificity of 92% and 65%, respectively [12]. This proves the role of elevated serum AMH in impaired folliculogenesis as well as granulosa cell function. Although increased AMH levels are predictive of increased ovarian response in women undergoing IVF [17], this does not apply for women with PCOS [18], however, conflicting results exist [19]. It was suggested that the increased levels of FSH during induction of ovulation in women with PCOS would relieve the inhibition of folliculogenesis due to elevated AMH levels allowing the development of mature follicles [20]. Clomiphene citrate results in increased serum FSH levels but not to the extent to inhibit the effect of AMH on folliculogenesis as the aim is to induce monofolicular growth resulting in cases of CC resistance, the contrast occurs in IVF cycles [12]. Also, multiple patient-related factors affected ovarian response with fasting insulin the best marker reported [15]; however; these factors were not assessed in the current study.

STRENGTHS AND LIMITATIONS OF THE STUDY

The study evaluated the basal characteristics of PCOS. The relation between serum AMH and ovarian response to CC was evaluated, however; larger sample size would be more informative. The effect of different methods of induction and its relation to serum AMH levels were not evaluated.

CONCLUSION

Serum AMH levels correlated with the patients' characteristics specific for PCOS. There was a significant decrease in serum AMH levels in responders than non-responders.

CONFLICTS OF INTEREST

None

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