



Anti-mullerian Hormone Serum Level as a Predictor of Responsiveness to Clomiphene Citrate Therapy in Iraqi Women with PCOS

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Abstract

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BACKGROUND: Ovulation induction in polycystic ovarian syndrome is one of the most common interventions used in the treatment of infertility and prediction of the response is very important. Although the serum anti-mullerian hormone (AMH) concentrations are known to be a reliable predictor of ovarian response in *in vitro* fertilization, in women with polycystic ovary syndrome (PCOS) with a higher level of AMH ovulation induction by clomiphene citrate may be not enough to increase the level of follicle-stimulating hormone and reduce the intraovarian AMH to a level that compatible with the restoration of ovulation.

AIM: Our study aimed to identify those females who will respond to treatment and the starting dose of anti-mullerian hormone serum level as a predictor of responsiveness to clomiphene citrate therapy in Iraqi women with PCOS.

METHODS: 79 females with polycystic ovarian syndrome were included in the study. AMH serum concentrations were determined on the second or 3rd day of the early follicular phase of the menstrual cycle before initiating clomiphene citrate in the first treatment cycle.

RESULTS: The females were falling-out into two groups according to their response to treatment. The patient who ovulated had a significantly lower AMH level among responders 7.89 \pm 3.2 compared to non-responder 9.41 \pm 3.27. The women who responded at 50 mg dose had lower AMH 6.56 \pm 2.2 compared to women who responded at 100mg clomiphene citrate had AMH of 8.74 \pm 3 with optimum cutoff value for AMH is 6.25 to respond.

CONCLUSION: Serum AMH level measurement prior to treatment initiation in women with PCOS may be a useful method for predicting the outcome of clomiphene citrate treatment, and for each specific ethnic group, there is a cutoff value for AMH above which the response to clomiphene citrate is impaired.

Introduction

Infertility is the second most common reason for women of childbearing age to attend their general practitioner. As nearly every seven heterosexual couples, there is one couple that has conceiving problems. For pregnancy to occur, there must be sperm and egg with the good fertile ability [1]. Common causes of infertility in the female are ovulatory and tubal factors [2]. Ovulatory disorders are a prominent cause of infertility, with polycystic ovary syndrome (PCOS) being the most prevalent. PCOS has a frequency of between 5% and 15% in the general population, depending on many variables such as phenotype, race, and the categorization system employed [3]. Due to the broad range of diagnostic criteria used, prevalence estimates range from 2.2% to as high as 26%. When the Rotterdam criteria were used to diagnose PCOS, the prevalence was more than double what was seen when the National Institutes of Health criteria were employed. The prevalence of PCOS may be different according to ethnic background. For example, in comparison to

Caucasians, a higher prevalence is observed among females of South Asian origin, where the presentation of PCOS occurs at a younger age and has more severe symptoms [4]. The pathophysiology of PCOS seems to be due to multiple factors and polygenic [5]. There is controversy about the definition of this syndrome. Important features involve disturbances in the menstrual cycle, clinical or biochemical hyperandrogenism, and obesity [6]. There are many aspects other than ovarian aspects that contribute to the pathophysiology of PCOS, but ovarian dysfunction is central [7]. It has been recognized that females with PCOS have elevated serum levels of anti-mullerian hormone (AMH) [8]. AMH is one of the transforming growth factors- β superfamily of peptides and its production is mainly by granulosa cells of the developing preantral and early antral follicles (AFC). Numerous studies have illustrated that AMH concentration is closely associated with AFC number [9]. Therefore, raised circulating AMH level in females with PCOS has strongly correlated to the large number of AFC related to this syndrome. The correlation between elevated blood AMH levels in females with PCOS and chronic infertility over a long period of time has been

established, and seems to be connected to a variety of factors [10]. Initially, the activity of aromatase enzyme in human beings and mice granulosa cells is directly stopped by AMH. In addition, Durlinger demonstrated that directly cultivating an in a vitro mouse model with AMH halted the formation of follicles from the resting primordial follicles pool and inhibited follicle-stimulating hormone (FSH)-stimulated preantral follicle growth. In the presence of AMH, laboratory diagnostic studies on human granulosa cells show that the aromatase enzyme activity is inhibited and the follicular response to FSH is reduced [9]. Thus, increased AMH inhibits estradiol synthesis and the FSH action, interfering with the FSH/E2-induced feed-forward loop, which further increases the cellular FSH receptor count and allows follicles to grow and mature. In this manner, the idea that increased AMH is required for folliculogenesis may be developed [11]. There are many methods for PCOS patients to promote ovulation, which can ultimately result in a successful live delivery. Each of these therapies varies in terms of aggressiveness and efficacy. From lifestyle modifications to insulin sensitizing drugs, selective estrogen receptor modulators, aromatase enzyme inhibitors, and gonadotropins, to laparoscopic ovarian drilling and assisted reproductive methods, each method induces ovulation through a distinct mode of action. In many ovulatory women, the first-line therapy remains ovulation stimulation with antiestrogen medications [12]. Anti-estrogen therapy is effective in inducing ovulation in 73% of female patients, resulting in a 29% live birth rate [12]. Clomiphene citrate (CC) is a specific estrogen receptor modulator consisting of two isomers, *zu*-clomiphene, and *en*-clomiphene, with *zu*-clomiphene being the more physiologically active by inhibiting estrogen receptors in the brain and pituitary, the anterior pituitary produces more gonadotropins, resulting in the ultimate maturation of follicles [3]. Using the best available knowledge to recognize women who are still unovulatory despite anti-estrogen treatment can guide these women to other therapeutic methods such as exogenous gonadotrophins, laparoscopic ovarian surgery, and insulin-sensitizing agents or more complicated assisted reproductive technology procedures, particularly in patients with increased reproductive age [13], [14]. In addition, the method of recognizing the elements that affect the prognosis also allows an insight into the abnormalities in the ovaries and the pathophysiology of anovulation [14]. Our study aimed to identify those females who will respond to treatment and the starting dose.

Methods

This study is an observational cohort study was conducted in Al-Ramadi and Al-Qaiem cities in outpatient clinics from January 2020 to February 2021

after informed consent for all participants. 79 women were included with a diagnosis of polycystic ovarian syndrome according to the Rotterdam criteria (two out of three) 1-Oligoovulation and/or anovulation (i.e., oligomenorrhoe or amenorrhoea) 2-hyperandrogenism (clinical features and/or biochemical elevation of testosterone) 3-Polycystic ovaries assessed by ultrasound. The inclusion criteria include patients. 1-Age 20-40 2-both primary and secondary infertility, with exclusion criteria include 1-Age more than 40. 2-Contraindications to CC 3-history of ovarian surgery 4-Thyroid disease. 5-Adrenal disease. 6-Significant Hyperprolactinemia. 7-Psychological disease or patient with a stressful life event. After complete history and examinations, all patients who meet the inclusion criteria were seen during the early follicular phase of the menstrual cycle (day 2 or 3 after spontaneous or progestogen promotion of menstrual bleeding) where ultrasound scanning was done to detect the volume of the ovaries (milliliters) and counting the AFC, blood samples were collected before the start of the first cycle of therapy with CC to establish the baseline blood level of AMH. AMH was quantified using an AMH Gen II ELISA. The AMH II Gen ELISA is an enzymatically amplified two-site immunoassay. The measurement range (from a limit of detection to highest calibrator): 0.05 to approximately 22.5 ng/ml. For the precision, the intra-assay coefficients of variation were $\leq 10.3\%$ and the inter-assay coefficients of variation were $\leq 10.8\%$. Measurements of the levels of other hormones involving luteinizing hormone, FSH), testosterone (T), insulin, and progesterone were also done. The participants receive CC 50 mg for 5 days from the second to the 7th day of the menstrual cycle. The patient's response to treatment is determined by transvaginal ultrasound monitoring of follicular growth till the appearance of a follicle of 18 mm and subsequent rupture of the follicle. Patients were considered as responders when there is a follicle of 18 mm regardless of the dose of CC used. If the ovulation does not occur, the dose of CC is increased to 100 mg in the next menstrual cycle. The endpoint was when the first ovulation occurs and when the ovulation occurs, the dose remained unchanged in the subsequent cycles. All women in this research had at least three treatment cycles. Following ultrasound confirmation of ovulation, some patients received a progestational drug to support the luteal phase. Patients who ovulated during CC treatment, regardless of the dosage, were classified as responders. Three failed attempts to induce ovulation in three CC cycles despite maximal dosage stimulation (100 mg/d) were deemed CC-resistant anovulatory patient.

Table 1: The socio-demographic characteristics of the patients

Variables	Min	Max	Mean	SD
Age in Years	17	40	25.72	4.97
BMI kg/m ²	18.49	37.11	27.28	4.48
Infertility duration (years)	1	13	3.43	2.78
Dose	50	150	112.03	29.10
AMH level	2.7	16.0	8.58	3.32

Statistical analysis

The description-statistical analysis was performed using mean and standard deviation for numerical variables, frequent and percentage for categorical variables. The association between response status, age, body mass index (BMI), dose, and infertility duration was examined by the independent t-test. All analysis was performed using SPSS version 24.

Results

Seventy-nine an ovulatory women with PCOS were involved in this study. They obtained 172 treatment cycles of CC.

Table 2: The responder and non-responder

Respond state	n	%
Responder	42	53.2
Non-responder	37	46.8

Regarding the BMI was 27.28 so most of the women in this study were overweight.

The patients were grouped into two groups according to their response to CC.

Table 3: The association between Response status and age, BMI, dose, and infertility duration

Variables	Response		p-value
	No response Mean ± SD	Response Mean ± SD	
Age	25.84 ± 5.83	25.62 ± 4.14	0.874
BMI	26.21 ± 4.38	28.21 ± 4.40	0.04*
Dose	108.33 ± 24.48	116.22 ± 33.44	0.657
Duration	3.70 ± 3.08	3.20 ± 2.50	0.232

BMI: Body mass index, SD: Standard deviation.

There was a significant association of BMI between responder and non-responder with a p-value of 0.04 Of the 79 women included in a study of 6 (7.6%) women ovulated at 50 mg of CC. This increased to 42 (92.9%). When the dose was increased to 100 mg of CC, 37 (46.8%) remained anovulatory (Figure 1 and Tables 1-3).

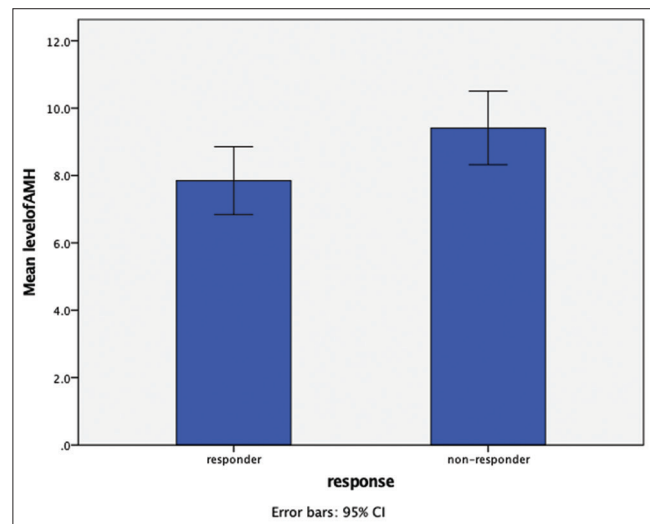


Figure 1: Mean anti-mullerian hormone level between responders and non-responders

Table 4: Mean AMH Level among responder and non-responder

Variable	Responder	Non-responder
	Mean ± SD	Mean ± SD
AMH Level	7.84 ± 3.23	9.41 ± 3.27

SD: Standard deviation, AMH: Anti-Mullerian hormone.

The patient who ovulated had a significantly lower AMH level among responders 7.84 ± 3.23 compared to non-responder 9.41 ± 3.27 (Table 4).

The women who respond at 50 CC had lower AMH 6.56 ± 2.2 compared to women who responded at 100 CC had AMH of 8.74 ± 3 (Figure 2).

Table 5: Cut-off value sensitivity, specificity, accuracy, the positive predictive value (PPV), and the negative predictive value

Variable	AUC	Optimum cut off value	Sensitivity	specificity	Accuracy	PPV	NPV
AMH	0.647	6.25	83.33%	34.69%	53.1%	43.86%	77.27%

PPV: Positive predictive value, AMH: Anti-mullerian hormone.

The area under the curve (AUC) is 0.647 with an optimum cutoff value for AMH is 6.25 for responders from non-responders (Table 5).

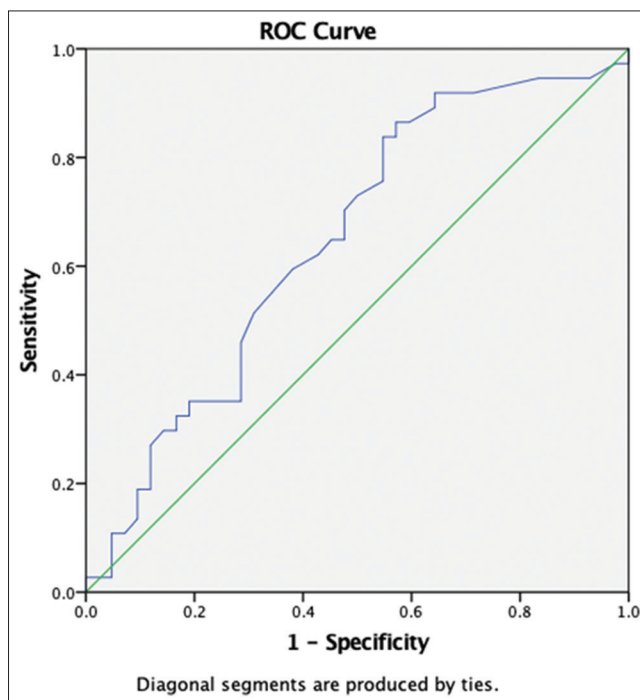


Figure 2: The optimum cut off value

Discussion

Although the serum AMH concentrations are known to be a reliable predictor of ovarian response in IVF, as high levels are regarded to anticipate an excessive ovarian response to gonadotropins treatment and decreased AMH level predicts diminished ovarian response to stimulation protocol [15]. In ovulation stimulation, oral medications such as oral CC produce

a mild increase in serum FSH; however, this may not be enough to reduce intraovarian AMH to a range that is consistent with ovulation restoration [16]. As a result, patients with higher AMH concentrations are more severely inhibited and are less likely to react to oral cc. PCOS is a major cause of anovulation in females and there are subgroups of patients with PCOS who have elevated levels of AMH that impairs the effect of FSH and participate in the pathophysiology of PCOS in that group [6]. AMH increases in PCOS and is responsible for impaired folliculogenesis. These observations suggest that the higher the AMH, the more impaired in disruption in folliculogenesis and less likely to respond to CC [17]. The study aims to identify this group of patients with high AMH levels. In addition, to find the specific cut of AMH beyond it, the response to CC is impaired in our Iraqi woman population as we supposed that this point is specific for each ethnic group. In this study, women who ovulated in response to CC had significantly lower AMH than women who did not ovulate in response to CC. compatible with the prior study of Maharan A and co-workers [18] which evaluates the effect of AMH on response rates of CC in sixty females with PCOS and found high circulatory AMH negatively correlated with the chance of ovulation and with the previous study of Wenyan who evaluates the effect of AMH level on the success of CC therapy in 81 anovulatory with PCOS and proves that with high AMH are more likely to not respond to cc treatment [19] and also with the study of Gülşen *et al.*, who found that serum AMH level was seen to be useful in the prediction of CC resistance with a sensitivity of 66% and a specificity of 89% when the threshold for AMH concentration was >12.38 ng/ml in PCOS patients [20]. The rate of response to CC in our study is 53%. This is lower than the expected rate of ovulation for CC, which is written in most textbooks by 70–80%. This can be illustrated by the reality that most of the women in the sample of our study were overweight. We have a cutoff point of 6.6 to predict no ovulation beyond this value with a sensitivity of 83% and specificity of 43% and a negative predictive value of 77.27%. We compared the value of the cutoff of AMH in our study around the population of Iraqi women to the cut-off value in other studies. In our study, we found the level of 6.6 ng/ml and it is twice that of the previous study of Maharan *et al.* who reported a cut-off value of 3.4 ng/ml [18] and it is approximate to the cutoff value reported by Wenyan found a cutoff value of 7.7 [19]. We suppose that the cut-off value differs from one community to another, which can be explained by the effect of genetic and environmental factors that influence the level of AMH and response to treatment. Further, different kits that are used for the measurement of AMH levels might result in considerable variation in serum AMH, so each community may have a specific AMH of its own. The dose utilized had a maximum of 100mg, as more than that (150 mg) is not recommended by the US Food and

Drug Administration. In this investigation, we used no more than 100 mg of CC.

Conclusion

The measurement of blood AMH levels in females with PCOS before therapy may be a useful tool for predicting the effect of CC treatment. This could be useful in counseling PCOS patients about the expected outcome of CC treatment, as well as making the ovulation induction protocol more customized and cost-effective for the patient by starting ovulation induction for those with high levels of AMH with either high dose CC or chronic low dose gonadotrophins.

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